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(54) **Compositions and method for recombinant production of crotalidus venom fibrolase.**

(57) Recombinant DNA encoding several forms of southern copperhead fibrolase, yeast expression vectors containing such DNA, and the production of southern copperhead fibrolase in yeast are disclosed.

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COMPOSITIONS AND METHODS FOR RECOMBINANT PRODUCTION OF CROTALIDUS VENOM FIBROLASE

Technical Field

This invention is in the fields of enzyme chemistry, genetic engineering, and thrombolytic therapy. More particularly, it relates to the recombinant production of the fibrinolytic enzymes, present in the venom of snakes of the family Crotalidae, such as Agkistrodon contortrix contortrix (southern copperhead) fibrolase.

Background Art

Thrombolytic therapy is an established procedure for treatment of various thromboembolic conditions such as pulmonary embolism, thrombophlebitis, and arterial thromboembolism. Many of the thrombolytic agents being investigated act indirectly by activating plasminogen throughout the circulation and are, therefore, not directed specifically towards the thrombus. These plasminogen-activating fibrinolytic agents have perceived disadvantages in therapy, such as inducing sufficient plasmin to deplete clotting factors to levels that enhance the probability of hemorrhagic complications after thrombolytic therapy. These perceived problems have led to the investigation of direct-acting thrombolytic agents, such as the fibrinolytic enzymes present in many snake venoms.

Numerous direct acting fibrinolytic enzymes have been identified in snake venoms. Among those isolated from Crotalidus venom are southern copperhead fibrolase, the purification and characterization of which is described in U.S. patent No. 4,610,879 and green pit viper fibrolase described in EPA publication no. 0020780. Preliminary investigation of southern copperhead fibrolase indicates it offers promise as a safe, effective, direct-acting agent for thrombolysis. Despite the therapeutic promise of this agent, however, it will be impractical to carry out extended clinical investigation of native fibrolase because of the scarcity of the source venom. Also fibrolase purified from venom pooled from a number of snakes is likely to be heterogenous due to allelic/polymorphic variation between individual snakes. A principal purpose of the present invention is to provide a more practical means for obtaining homogenous Crotalidus fibrolases than purifying them from venom.

Disclosure of the Invention

Accordingly, one aspect of the invention is recombinant DNA encoding a Crotalidus fibrolase, such as southern copperhead fibrolase.

Cloning and expression vectors containing such recombinant DNA are another aspect of the invention.

Hosts such as transformed bacteria, yeast, and mammalian cells which contain such expression vectors and are capable of producing active recombinant Crotalidus fibrolase are another aspect of the invention.

Methods for producing Crotalidus fibrolase which employ such hosts are still another aspect of the invention.

Recombinant homogeneous Crotalidus fibrolase which is free of other Crotalidus proteins is a further aspect of the invention.

Brief Description of the Drawings

Figure 1 shows the nucleotide sequence and the deduced amino acid sequence of a southern copperhead fibrolase clone designated SVG-48 (or FIB 48).

Figure 2 shows the partial nucleotide sequence and the deduced partial amino acid sequence of another southern copperhead fibrolase clone designated SVG-51 (or FIB 51).

Figure 3 shows a comparison of the nucleotide sequences of the coding strands of the fibrolases of Figures 1 and 2. Nucleotide variances between the sequences are asterisked.

Figure 4 shows a comparison of the amino acid sequences (using single letter amino acid designations) of the fibrolases of Figures 1 and 2. Amino acid variances between the two sequences are asterisked.

Figure 5 is a flow diagram depicting a scheme for preparing the yeast expression plasmids pAB24F248 or pAB24F251 which can be used to produce southern copperhead fibrolase in yeast.

Figure 6 shows the nucleotide sequence of the 1341 bp ADH2-GAPDH promoter fragment referred to in the Examples.

Figure 7 shows the nucleotide sequence and amino acid sequence of an α-factor profibrolase fusion construct with a Lys-Arg processing site at the pro-mature junction (Example 5, infra).

Figures 8, 9, 10 and 11 are the nucleotide and corresponding amino acid sequences of the α-factor profibrolase fusion constructs of plasmids pKS308, pKS311, pKS314 and pKS317 of Example 5, infra.

Modes for Carrying Out the Invention

1. Definitions

The term "recombinant" as used herein to characterize DNA encoding Crotalidus fibrolase intends DNA of genomic, cDNA, semisynthetic, or synthetic origin which, by virtue of its origin or manipulation is (1) not associated with all or a portion of the DNA with which it is associated in nature or in the form of a library and/or (2) linked to DNA other than that to which it is linked in nature. "Recombinant" as used to describe Crotalidus fibrolase intends protein produced from such DNA.

A "replicon" is any genetic element (e.g., a plasmid, a chromosome, a virus) that behaves as an autonomous unit of polynucleotide replication within a cell; i.e., capable of replication under its own control.

A "vector" is a replicon in which another polynucleotide segment is attached, so as to bring about the replication and/or expression of the attached segment. An "expression vector" refers to a vector capable of autonomous replication or integration and contains control sequences which direct the transcription and translation of the southern copperhead fibrolase DNA in an appropriate host.

A "coding sequence" is a polynucleotide sequence which is transcribed and/or translated into a polypeptide.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase and initiating transcription of a downstream (i.e., in the 3′ direction) coding sequence.

A coding sequence is "under the control" of the promoter sequence in a cell when transcription of the coding sequence results from the binding of RNA polymerase to the promoter sequence; translation of the resulting mRNA then results in the polypeptide encoded within the coding sequence.

"Operably linked" refers to a juxtaposition wherein the components are configured so as to perform their usual function. Thus, control sequences operably linked to a coding sequence are capable of effecting the expression of the coding sequence.

"Control sequences" refers to those sequences which control the transcription and/or translation of the coding sequence(s); these may include, but are not limited to, promoter sequences, transcriptional initiation and termination sequences, and translational initiation and termination sequences. In addition, "control sequences" refers to sequences which control the processing of the polypeptide encoded within the coding sequence; these may include, but are not limited to sequences controlling secretion, protease cleavage, and glycosylation of the polypeptide.

"Transformation" is the introduction of an exogenous polynucleotide into a host cell. The exogenous polynucleotide may be maintained as a plasmid, or alternatively, may be integrated within the host genome.

"Crotalidae" denotes the family of snakes commonly known as pit vipers. Members of the Crotalidae family include species of the subfamilies Agkistrodon (A), Crotalus (C), Bothrops (B) and Trimeresurus (T). Examples of snake species of this family are A. acutus, A. bilineatus, A. caliginosus, A. contortrix, A. halys, A. halys blomhoffii, A. hypnale, A. mokasen, A. piscivorous, A. rhodostoma, A. saxatilis, B. alternatus, B. atrox, B. bilineatus, B. caribbaeus, B. godmani, B. itapetiningae, B. jararaca, B. jararacussu, B. lanceolatus, B. lansbergii, B. nasuta, B. neuwiedi, B. nigroviridis marchi, B. nummifer (B. nummifera), B. schlegelii, C. adamanteus, C. atrox, C. basiliscus, C. cerastes, C. confluentus, C. durissus, C. durissus terrificus, C. horridus, C. lepidus, C. mitchellii, C. molossus, C. ruber, C. scutulatus, C. tigris, C. unicolor, C. viridis lutosus, C. viridis oreganus, L. mutus, S. catenatus, S. miliarius, S. ravus, T. albolabris, T. elegans, T. flavoviridis, T. gramineus, T. monticola, T. mucrosquamatus, T. okinavensis, T. popeorum, T. purpureomaculatus, T. stejnegeri, T. tokarensis, T. wagleri.

2. Recombinant Southern Copperhead DNA

Figures 1-4 show the nucleotide sequences and deduced amino acid sequences of two southern copperhead fibrolase clones, designated pSVG-48 (or Fib 48) and pSVG-51 (or Fib 51). Fib 48 includes the complete sequence for southern copperhead preprofibrolase whereas Fib 51 lacks a short 5′ sequence and initiator codon.

Based on the Fib 48 and Fib 51 sequences and amino acid sequences it is apparent that there are polymorphic variations and/or allelic variations of southern copperhead fibrolase. Amino acid sequencing of native protein shows different residues at amino acids 210 (Tyr-----Asn) and 360 (Thr/Val-----Met) (Figure 4) from the sequences predicted from the two clones thus providing further evidence of such variation.

Further amino acid sequencing of native protein provided an amino acid sequence for the mature fibrolase protein differing by two amino acids from the sequence predicted from Fib 51 (Figures 2 and 4). The six differences are (numbering begins at amino acid 185 of Figure 2): 123 (Pro-----Ser) and 170 (Val-----Met).

This sequence is sometimes referred to herein as the "corrected native sequence".

It is further expected that fibrolases from other Crotalidus species may also vary intraspecies as well as varying from species to species but will exhibit significant homology (i.e., 30% or more identity, more usually 50% or more identity, in amino acid sequence to the southern copperhead fibrolase sequences described herein). Fibrolase DNA of Crotalidae of species other than southern copperhead fibrolase may be identified and isolated as described below. Amino acid sequences may be deduced from that DNA.

The recombinant southern copperhead DNA of the invention encodes at least amino acids 192 to 393 of the Fib 48 sequence of Figure 4, or the corresponding amino acids of the Fib 51 sequence of Figure 4 (amino acids 186-387 of the Fib 51 sequence), or the corrected native amino acid sequence shown in Figure 7, and analogs of those amino acid sequences which are substantially homologous and functionally equivalent thereto. Based on the sequences of clones pSVG-48 and pSVG-51 shown in Figures 1 and 2 and amino acid sequencing of native southern copperhead fibrolase the structure of southern copperhead fibrolase is believed to be that of a prepropolypeptide with the first 190-191 amino acids of the Fib 48 sequence being a leader sequence and the mature protein beginning at the Gln at position 190 or the Gln at position 191 (Figure 1) or at 185/186 in the Fib 51 sequence (Figure 2). It is further believed that the carboxy terminus of the prepropolypeptide is processed to remove the final 18 amino acids. Mass spectrophotometric analysis of peptides derived from mature native southern copperhead fibrolase indicates the amino terminus is a cyclized glutamine residue with some molecules starting at the Gln at 185 (i.e., pGlu-Gln-Arg/Phe-...) and other molecules starting at the Gln at 186 (i.e., pGlu-Arg/Phe-...) of Figure 2. Reaction of mature native fibrolase with DTNB indicated there are no free sulfhydryl groups in southern copperhead fibrolase meaning there are three disulfide bonds in the molecule. The term "substantially homologous" as used herein intends to include such variations polymorphic and/or allelic variations as well as other variations that do not destroy the fibrolase activity of the molecule. In general, the homology in amino acid sequence will be at least about 70%, more usually at least about 75%. The term "functionally equivalent" intends that the sequence of the analog defines a protein having the biological activity of fibrolase (as measured by the azocasein or fluorometric assays described in the examples). The sequence may include a portion or all of the leader sequence (amino acids 1-190/191 and all or a portion of the processed carboxy terminus of the prepropolypeptide (amino acids 393-411 of Fib 48 or the corresponding amino acids of Fib 51).

The recombinant fibrolase DNA may be genomic, cDNA or synthetic DNA. By way of example, the sequences shown in Figures 1 and 2 were obtained from a cDNA library prepared from mRNA obtained from southern copperhead venom glands. The library was screened with cDNA probes based on abundant mRNA sequences and clones showing strong hybridization were selected. Identified fibrolase clones may in turn be used to screen genomic or cDNA southern copperhead libraries to obtain allelic or polymorphic variants of the fibrolase genes shown in the drawings or to screen libraries of other Crotalidus species to identify the fibrolase genes contained therein. A DNA sequence encoding the "native" protein sequence may be made by site specific mutagenis of clone 51 cDNA or by other conventional methods. The general procedures used to prepare probes based on the identified clones, to prepare other libraries, and to screen those libraries for Crotalidus fibrolase sequences are known in the art and do not require elaboration. Based on the amino acid sequences deduced from the illustrated DNA sequences, synthetic genes encoding fibrolase may be prepared in vitro by synthesizing individual overlapping complementary oligonucleotides and filling in single stranded nonoverlapping portions using DNA polymerase in the presence of the deoxyribonucleotide triphosphates. For expression in a particular organism, it may be desirable to use a synthetic DNA sequence that employs codons preferred by the particular host in which the DNA is expressed. Mutations of the genes may be made by site specific mutagenesis. Such mutations may be used to make fibrolase analogs.

3. Cloning of Fibrolase DNA

The fibrolase DNA can be cloned into any suitable replicon to create a vector, and thereby be maintained in a composition which is substantially free of vectors that do not contain the fibrolase gene (e.g., other clones derived from the library). Numerous cloning vectors are known to those of skill in the art, and the selection of an appropriate cloning vector is a matter of choice. Examples of vectors for cloning and host cells which they can transform include the bacteriophage λ (E. coli), pBR322 (E. coli), pACYC 177 (E. coli), pKT230 (gram-negative bacteria), pGV1106 (gram-negative bacteria), pLAFR1 (gram-negative bacteria), pME290 (non-E. coli gram-negative bacteria), pHV 14 (E. coli and Bacillus subtilis), pBD9 (Bacillus), pIJ61 (Streptomyces), pUC6 (Streptomyces), actinophage ϕC31 (Streptomyces), YIp5 (Saccharomyces), YCp19 (Saccharomyces), YEp24 and YEp13 (Saccharomyces), and bovine papilloma virus (mammalian cells).

4. Expression of Fibrolase DNA

The polynucleotide sequence encoding the fibrolase polypeptide is expressed by inserting the sequence into an appropriate replicon thereby creating an expression vector, and introducing the resulting expression vector into a compatible host.

In creating an expression vector the sequence encoding the fibrolase polypeptide is located in the vector with the appropriate control sequences. The positioning and orientation of the coding sequence with respect to the control sequences is such that the coding sequence is transcribed under the control of the control sequences: i.e., the promoter will control the transcription of the mRNA derived from the coding sequence; and the ribosomes will bind at the ribosomal binding site to begin the translational process; and the stop codon used to terminate translation will be upstream from the transcriptional termination codon. Commonly used prokaryotic control sequences include such commonly used promoters as the β-lactamase (penicillinase) and lactose (lac) promoter systems (Chang et al, Nature (1977) 198:1056) and the tryptophan (trp) promoter system (Goeddel et al, Nucleic Acids Res (1980) 8:4057) and the lambda-derived P_L promoter and N-gene ribosome binding site (Shimatake et al, Nature (1981) 292:128). Control sequences for yeast vectors include promoters for the synthesis of glycolytic enzymes (Hess et al, J Adv Enzyme Reg (1968) 7:149; Holland et al, Biochemistry (1978) 17:4900). Additional promoters known in the art include the promoter for 3-phosphoglycerate kinase (Hitzeman et al, J Biol Chem (1980) 255:2073). Other promoters, which have the additional advantage of transcription controlled by growth conditions and/or genetic background are the promoter regions for alcohol dehydrogenase 2 (ADH2), isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, the alpha factor system and enzymes responsible for maltose and galactose utilization. It is also believed transcription terminator sequences are desirable at the 3′ end of the coding sequences. Such terminators are found in the 3′ untranslated region following the coding sequences in yeast-derived genes. Expression vectors for mammalian cells such as VERO, Hela or CHO cells, ordinarily include promoters and control sequences compatible with such cells as, for example, the commonly used early and late promoters from Simian Virus 40 (SV40) (Fiers et al, Nature (1978) 273:113), or other viral promoters such as those derived from polyoma, Adenovirus 2, bovine papilloma virus, or avian sarcoma viruses. The controllable promoter, hMTII (Karin, M., et al, Nature (1982) 299:797-802) may also be used.

In addition to control sequences, it may be desirable to add regulatory sequences which allow for regulation of the expression of the fibrolase gene relative to the growth of the host cell. Examples of regulatory systems are those which cause the expression of a gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. In prokaryotic systems these would include the lac and trp operator systems. In eukaryotic systems induction can occur in methallothionein genes with heavy metals and the Mouse Mammary Tumor Virus (MMTV) system with steroids. In these cases, the sequence encoding the fibrolase polypeptide would be placed in tandem with the regulatory element.

There are also selective elements which give rise to DNA amplification which in turn can result in higher levels of specific protein production. In eukaryotic systems these include the dihydrofolate reductase gene (dhfr) which is amplified in the presence of methotrexate, and adenosine deaminase (ADA) in the presence of deoxycorfomycin. In these cases the sequence encoding the fibrolase polypeptide may either be present on the same plasmid or merely be cotransfected together with the selectable element to allow for integration within the host cell genome near each other.

Other types of regulatory elements may also be present in the vector, i.e., those which are not

necessarily in tandem with the sequence encoding fibrolase. An example is the SV40 enhancer sequence, which, by its mere presence, causes an enhancement of expression of genes distal to it.

Modification of the sequence encoding fibrolase, prior to its insertion into the replicon, may be desirable or necessary, depending upon the expression system chosen. For example, in some cases, it may be necessary to modify the sequence so that it may be attached to the control sequences with the appropriate orientation, i.e., to maintain the reading frame. In some cases, it may be desirable to add sequences which cause the secretion of the polypeptide from the host organism, with subsequent cleavage of the secretory signal. The techniques for modifying nucleotide sequences utilizing cloning are well known to those skilled in the art. They include, e.g., the use of restriction enzymes, of enzymes such as Bal31 to remove excess nucleotides, and of chemically synthesized oligonucleotides for use as adapters, to replace lost nucleotides, and in site directed mutagenesis.

The appropriately modified sequence encoding the fibrolase polypeptide may be ligated to the control sequences prior to insertion into a vector. Alternatively, the coding sequence can be cloned directly into an expression vector which already contains the control sequences and an appropriate restriction site. For expression of the fibrolase polypeptide in prokaryotes and in yeast, the control sequences will necessarily be heterologous to the coding sequence. In cases where the fibrolase gene is to be expressed in cell lines derived from vertebrates, the control sequences may be either heterologous or homologous, depending upon the particular cell line.

In experimentation with various expression vectors--integrated, nonintegrated, intracellular (internal), extracellular (secretory)--carried out to date, yeast expression vectors in which the S. cerivisiae α-factor leader sequence directs secretory expression of a profibrolase gene encoding the corrected native fibrolase sequence and having an Lys Arg (KR) processing site at the pro-mature junction of the profibrolase coding region have given the highest yields of active mature fibrolase. Constructs using both nonregulatable promoters (e.g., GAPDH) and regulatable promoters (e.g. ADH2/GAPDH) were made. Use of a regulatable promoter may be desirable to overcome possible toxic effects from large amounts of fibrolase during cell growth. A summary of these constructs is provided in the Examples, infra.

Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described by Cohen, S.N., Proc Natl Acad Sci (USA) (1972) 69:2110, or the $RbCl_2$ method described in Maniatis et al, Molecular Cloning: A Laboratory Manual (1982) Cold Spring Harbor Press, p. 254 and Hanahan, D., J Mol Biol (1983) 166: 557-580 may be used for prokaryotes or other cells which contain substantial cell wall barriers. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, Virology (1978) 52:546, optionally as modified by Wigler, M., et al, Cell (1979) 16:777-785 may be used. Transformations into yeast may be carried out according to the method of Beggs, J.D., Nature (1978) 275:104-109 or of Hinnen, A., et al, Proc Natl Acad Sci (USA) (1978) 75:1929 or Ito et al, J Bacteriology 153:163.

Transformed cells are then grown under conditions which permit expression of the fibrolase gene and, if appropriate, processing into the mature protein. Because the gene is expressed in heterologous organisms/cells (i.e., not snake), the protein is free of the other snake proteins with which it is associated in southern copperhead venom. This recombinant fibrolase is also homogeneous and lacks allelic/polymorphic variations typically found in protein isolated from pooled snake venom. The thus synthesized recombinant fibrolase is then isolated from the host cells and purified. If the expression system secretes the fibrolase into the growth media, the fibrolase is isolated directly from the media. If the recombinant fibrolase is not secreted, it is isolated from cell lysates. The selection of the appropriate growth conditions and recovery methods are within the skill of the art. With regard to purification, see for instance, U.S. Patent No. 4,610,879.

5. Use and Administration of Recombinant Fibrolase

Recombinant fibrolase prepared according to the invention may be formulated and administered to vertebrates, particularly mammals including humans, for treatment of thromboembolic conditions in the same manner as fibrolase isolated from venom (see U.S. Pat. No. 4,610,879 and EPA Publication No. 0020780 in this regard). The fibrolase will typically be formulated with a pharmaceutically acceptable injectable carrier such as physiological saline, Ringer's solution and the like for injection into circulation. Mildly hydrophilic polymeric substances such as albumins, polyvinylalcohol, polyethylene glycol, and the like may be added to stabilize the fibrolase. The concentration of fibrolase in the injectable will normally be in the range of 0.01 to 1 mg/ml. The patient will normally be infused with a dose of about 0.1 to 80 mg fibrolase for adult humans.

Examples

The following examples further describe the isolation of DNA encoding fibrolase and the expression of that DNA in yeast to produce recombinant fibrolase.

In the following, "digestion" refers to the enzymatic cleavage of DNA by restriction endonucleases. Restriction endonucleases commonly referred to as restriction enzymes are well characterized and commercially available and used in accordance with manufacturer's specifications. Digestion with restriction enzymes is frequently followed by treatment with alkaline phosphatase according to manufacturer's specifications to remove the terminal 5′ phosphates, thus preventing self ligation of a vector having two compatible ends.

"Fill in" refers to the enzymatic process of creating blunt ends by repairing overhanging ends generated by certain restriction enzymes. The repair is a DNA polymerase I large fragment (Klenow) and deoxynucleotide triphosphates and is used according to manufacturer's specifications.

Gel isolation of a DNA restriction fragment refers to the recovery of a specific fragment, electrophoretically separated on either an agarose gel or polyacrylamide gel (depending on size of fragment), by either electroelution or melting and extraction of gel slice.

All DNA manipulations are done according to standard procedures. See Maniatis et al, Molecular Cloning, Cold Spring Harbor Lab., 1982. All enzymes used are obtained from commercial sources and used according to the manufacturer's specifications.

1. RNA Isolation from Southern Copperhead Venom Glands

The method used to isolate RNA from snake venom glands utilizes guanidinium thiocyanate as a chaotropic agent during tissue homogenization, direct precipitation of RNA from the guanidinium solution by LiCl followed by purification of the RNA from residual DNA and protein by successive urea-LiCl washes.

Southern copperhead snakes (Agkistrodon c. contortrix) were obtained from Zooherp, Inc. Three snakes were killed with a lethal dose of Nembutol and the six venom glands were dissected free of the surrounding muscle. They were frozen in liquid nitrogen and the pulverized tissue was homogenized in 8 ml of lysis buffer containing 5 M guanidinium thiocyanate, 100 mM Tris•HCl (pH 7.5), 10 mM EDTA (pH 8.0) and 14% (v/v) beta-mercaptoethanol using a Tekmar Tissumizer for one min at room temperature. After dissolution, the homogenate was clarified by centrifugation (10,000 X g, 4° C, 10 min). The RNA was precipitated (16-24 hr, 4° C) by adding 5.5 volumes of 4 M LiCl. The RNA and some protein and DNA was then pelleted by centrifugation (10,000 X g, 4° C, 30 min). The pellet was mechanically resuspended in 5 ml of a solution of 3 M LiCl containing 4 M urea by using a pasteur pipet sealed at the end, and then vortexed vigorously for about one min. The volume of the suspension was adjusted to 30 ml and the precipitate collected by centrifugation as described above. This procedure was repeated 2 times. The RNA pellet was finally dissolved in 2.5 ml of 1% sodium dodecyl sulfate (SDS). One volume of phenol (equilibrated with 50 mM Tris•HCl, pH 8.0, 1 mM EDTA and 0.1% beta-mercaptoethanol) was added and the mixture vortexed vigorously for about one min. An equivalent volume of chloroform:isoamyl alcohol (24:1) was added and the solution vortexed one min. The mixture was centrifuged (4,000 X g, 4° C, 20 min); the aqueous phase was extracted with chloroform:isoamyl alcohol and centrifuged (4,000 X g, 4° C, 10 min). The RNA was precipitated (16-24 hr, -20° C) from the aqueous phase by adding 1/10 volume of 2 M potassium acetate (pH 5.0) and 3 volumes of absolute ethanol. The precipitated RNA was collected by centrifugation (10,000 X g, 4° C, 30 min) and dissolved in 0.5 ml of diethylpyrocarbonate-(DEP)-treated water and poly(A)+ RNA was prepared as described by Maniatis et al. The concentration of the poly(A)+ RNA was calculated based on an extinction coefficient at 260 nm of 25 per mg of RNA. The RNA was precipitated as described above and stored at -20° C.

2. Construction of cDNA Library from Southern Copperhead Venom Gland RNA

Double-stranded cDNA was prepared from Southern copperhead venom gland poly(A)+ RNA essentially as described by Gubler, U. and Hoffman, B.J., Gene (1983) 25:263-269. After methylation of the internal EcoRI sites and the addition of EcoRI linkers, the cDNA was ligated into the EcoRI site of λgt10. These steps are described in detail below:

A. For first strand synthesis, 3 µg of poly(A)+ RNA in 6 µl of DEP-treated water was used in a total volume of 20 µl. The final mixture contained 2 µl of 0.1 M dithiothreitol (DTT), 1 µl of RNasin (Promega Biotec, diluted to 10 units/µl in 10 mM DDT), 2 µl of 0.1 M $MgCl_2$, 1 µl of 1M Tris•HCl (pH 8.3), 2 µl of 1 mg/ml oligo(dT)12-18 (P-L Biochemicals), 2 µl of 10 mM each d(ACGT)Tps (P-L Biochemicals), 1 µl of [^{32}p] dCTP (Amersham, 3000 Ci/mmole), 1 µl of 80 mM sodium pyrophosphate and 2 µl of reverse transcriptase (Molecular Genetics Resources). The reactants were combined on ice, incubated for 2 min at room temperature and then for 60 min at 45° C. The reaction was stopped by adding 2 µl of 0.5 M EDTA (pH 7.0) and then phenol/chloroform/isoamyl alcohol (25/24/1) extracted. The organic phase was extracted with an equal volume of TE (10 mM Tris•Hcl, pH 8.0, 1 mM EDTA), the aqueous phases were combined and the first strand cDNA was precipitated by adding 16 µl of 7.5 M ammonium acetate and 120 µl of absolute ethanol. The pellet of precipitated nucleic acid was collected by microcentrifugation, dried in a Savant Speed-Vac Concentrator, dissolved in 50 µl of H_2O and precipitated by adding 20 µl of 7.5 M ammonium acetate and 150 µl of absolute ethanol. This procedure was repeated a third-time, the pellet was rinsed in absolute ethanol and dissolved in 71 µl of DEP-treated water.

B. For second strand cDNA synthesis, 71 µl of first-strand cDNA was used in a total volume of 100 µl that included 2 µl of 1 M Tris•Hcl (pH 7.5), 5 µl of 100 mM $MgCl_2$, 1 µl of 1 M ammonium sulfate, 10 µl of 1 M KCl, 1.25 µl of 4 mg/ml bovine serum albumin, 5 µl of 1 mM each of d(ACGT)TPs, 1 µl of [^{32}P]-dCTP (Amersham, 3000 Ci/mmole), 1.5 µl of RNase H (Bethesda Research Laboratories) and 2.3 µl (23 units) of DNA polymerase I (New England Biolabs). The reaction was incubated for 1 h at 14° C and then for 1 h at room temperature. It was terminated by the addition of 4 µl of 0.5 M EDTA (pH 7.0) and extracted with an equal volume of phenol/chloroform/isoamyl alcohol. The double-stranded cDNA was precipitated by the addition of 40 µl of 7.5 M ammonium acetate and 300 µl of absolute ethanol. The pellet was reprecipitated two times as described in step I. Finally, the pellet of cDNA was rinsed in absolute ethanol, dried and dissolved in 25 µl of DEP-treated water.

C. To fill in the ends of the cDNA so that chemically synthesized linkers could be ligated to them, 7.7 µl of DEP-treated water was added to the 25 µl of double-stranded cDNA. The final reaction volume was 50 µl and included 5 µl of (0.5 M Tris•HCl, pH 8.0, 60 mM $MgCl_2$ and 250 mM KCl), 5 µl of 10 mM DTT, 5 µl of 1 mM each d(ACGT)TPs, 1.3 µl of 4 mg/ml BSA and 1 µl (5 units) of T4 DNA polymerase (Bethesda Research Laboratory). The reaction was incubated at 37° C for 30 min and terminated by the addition of 2 µl of 0.5 M EDTA (pH 7.5). The solution was heated for 10 min at 70° C and the nucleic acid precipitated by the addition of 20 µl of 7.5 M ammonium acetate and 150 µl of ethanol. The precipitate was collected, reprecipitated once, rinsed and dried as described in step I. The DNA was dissolved in 27.5 µl of TE.

D. To protect internal EcoRI sites within the DNA from subsequent cleavage, the DNA was modified by EcoRI methylase. To 27.5 µl of DNA was added 10 µl of 5X buffer (0.5 M Tris•Hcl, pH 8.0, 5 mM EDTA), 5 µl of 4 mg/ml BSA, 5 µl of 100 µM SAM (S-adenosyl-L-methionine, Sigma) and 2.5 µl (50 units) EcoRI methylase (New England Biolabs). The reaction was incubated at 37° C for 30 min and then heated at 70° C for 10 min. The methylated cDNA was precipitated by adding 20 µl of 7.5 M ammonium acetate and 150 µl of absolute ethanol. The precipitate was collected, reprecipitated twice, rinsed and dried as described in step A.

E. EcoRI dodecamer linkers were kinased in a total volume of 50 µl as follows: 15 µl (1.5 mmoles) of linker solution (Collaborative Research), 5 µl of 10X kinase buffer (700 mM Tris•Hcl, pH 7.5, 100 mM $MgCl_2$, 50 mM DTT), 5 µl of 10 mM ATP, 22 µl of H_2O and 3 µl (15 units) of T4 polynucleotide kinase (New England Biolabs). The reaction was incubated at 37° C for 2 h.

F. To ligate the kinased EcoRI linkers to the methylated cDNA, 10 µl of water was added to the dried cDNA from step D followed by 10 µl of linkers from step E, 1.5 µl of 10 mM ATP, 1.5 µl of 10X ligase buffer (0.5 M Tris•Hcl pH 8.0, 0.1 M MgCl2, 0.2 M DTT) and 2 µl of T4 DNA ligase (New England Biolabs). The total reaction of 25 µl was incubated at 14° C overnight.

G. The cDNA, the T4 DNA ligase was inactivated by heating the reaction from step F for 15 min at 70° C. To the mixture was added 70 µl of water, 10 µl of 10X TMN (100 mM Tris•Hcl, pH 7.5, 100 mM $MgCl_2$ and 1.0 M NaCl) and 5 µl (100 units) of EcoRI. The 100 µl reaction was incubated at 37° C for 3 h to remove excess linkers from the cDNA and then phenol/chloroform/isoamyl alcohol extracted. One-tenth volume of 5 M NaCl was added to the aqueous phase and the "trimmed" cDNA was fractionated from excess linkers by gel filtration on a 0.7 X 18 cm Sepharose CL4B column. The elution buffer was 0.4 M NaCl, 10 mM Tris•Hcl, pH 8.0, 1 mM EDTA. The radioactivity of the fractions was determined and the peak of cDNA pooled and precipitated one time with 2 volumes ethanol as described in step A then precipitated.

H. To prepare the λgt10 vector for cloning the cDNA, 25 µg of λgt10 was digested with EcoRI. The final reaction volume was 250 µl and contained 1X TMN and 100 units of EcoRI. The enzyme digestion was carried out at 37° C for 2 h and was then extracted with chloroform. The linearized λgt10 was precipitated one time with ethanol as described in step A. The dried DNA was dissolved in 100 µl of TE.

I. To ligate the vector DNA to the cDNA, 12 µl (3 µg) was added to the ethanol-precipitated EcoRI-linked cDNA (step G). The mixture was dried and 7 µl of water was added followed by 1 µl of 10X ligase buffer and 1 µl of 10 mM ATP. The mixture was incubated at 37° C for 5 min, chilled to 4° C and 1 µl of T4 DNA ligase was then added. After incubation at 14° C overnight, the ligation mixture was stored at -20° C.

J. To package the recombinant DNA from step I into phage particles and plate them using E. coli strain C600Δhfl as a host, one-third (3 µl) of the ligation mixture was treated using Gigapack-plus according to the manufacturer's (Stratagene) instructions. Two hundred thousand recombinant phage were obtained.

3. Screening of the Snake Venom Gland cDNA Library

One thousand recombinant phage from the southern copperhead snake venom gland cDNA library were plated onto Luria agar using E. coli host strain C600Δhfl according to the manufacturer's (Strategene) instructions. The transfer of phage DNA to nitrocellulose filters (phage lifts) and the subsequent hybridization of the filters with ^{32}P-labeled single stranded (first strand) cDNA probe were done essentially as described by Maniatis et al. This strategy was selected since fibrolase is an abundant protein in snake venom and, therefore, the mRNA encoding this enzyme is expected to also be abundant.

The plaque lifts were done in duplicate, and the filters preannealed overnight at 42° C in 50% formamide, 5% SSC, 2x Denhardt's, 50 mM sodium phosphate, pH 6.5, 0.25% SDS and 100 µg/ml denatured salmon testes DNA. The filters were then hybridized with 250,000 cpm/ml of the ^{32}P-labeled stranded cDNA probe (boiled for 10 min in 0.4 N NaOH) made from the poly(A)+ RNA using the procedure described previously. Hybridization was done at 42° C overnight in preanneal solution that also contained 10% (w/v) sodium dextran sulfate. Washing of the filters was done in 2x SSC, 0.1% SDS for 30 min at room temperature, followed by 30 min at 50° C. The autoradiogram was exposed with an intensifying screen at 80° C overnight.

Sixty of the strongly hybridizing plaques were removed by coring and the phage eluted into PSB (100 mM NaCl, 10 mM Tris, pH 7.5, 10 mM $MgCl_2$, 500 mg/l gelatin). By plating appropriate dilutions of the phage stock eluate and screening by hybridization as described, an isolated bacteriophage colony was obtained for six of the original plaque.

Lambda DNA was isolated from each clone and the EcoRI-EcoRI cDNA inserts were purified away from the vector.

Restriction digests were done according to manufacturer's (New England Biolabs) instructions on the EcoRI fragments using PstI and HindIII separately. The results of the restriction analyses are shown in Table 1. The restriction analyses show that of the 6 clones screened, there are 3 distinct classes. Two of these (SVG-48 and SVG-51) were completely sequenced. The sequences are shown in Figs 1 and 2, respectively.

Table 1

Clone	Eco RI Fragments (kb)	Pst I Fragments (kb)	Hind III Fragments (kb)
SVG-6	1.1	0.5-, 0.6+	1.1
SVG-8	1.8	0.2, 0.6, 1.1	0.4, 1.4
SVG-20	1.3	0.5, 0.8	1.3
SVG-23	1.8	0.2, 0.5-, 1.1	0.4, 1.4-
SVG-48	1.8	0.2, 0.6, 1.1	0.4, 1.4
SVG-51	1.8	0.2, 0.5, 1.1	0.4, 1.4

4. Intracellular Expression of Fibrolase Protein

The following describes the intracellular expression of the fibrolase protein in yeast under the control of the regulatable promoter ADH2-glyceraldehyde-3-phosphate-dehydrogenase (GAPDH or GAP) and GAPDH terminator.

A. Construction of a Yeast Expression Vector for Fibrolase:

The scheme used to prepare the yeast expression vector is shown in Figure 5.

SVG 48 (or SVG 51) was digested with StuI and PstI and the resulting 717 bp StuI/PstI fragment was gel isolated. That fragment was ligated to the HindIII/StuI and PstI/SalI synthetic adaptors shown in Figure 5. The resulting 742 bp SalI/HindIII fibrolase gene encoding 231 amino acids was ligated to a 1341 bp ADH2-GAPDH promoter fragment (Figure 6). The resulting ligation product was ligated into the plasmid pPGAP-1 which had been cut with BamHI and SalI. pPGAPI is a yeast expression cassette vector which has a polyrestriction site linker between the GAPDH terminator and a truncated GAPDH promoter region. The polyrestriction site contains the recognition sites for NcoI, EcoRI, and SalI, and the cassette is excisable as a BamHI fragment. The preparation of pPGAPI is described in EPO 0 164 556 and Travis, J., et al, J Biol Chem (1985) 260(7):4384-4389. In both references pPGAPI is referred to pPGAP. The resulting plasmid was called pBRF248 (or pBRF251).

pBRF248 (or pBRF251) was digested with BamHI and the resulting BamHI cassette containing the ADH2-GAPDH promoter, fibrolase structural gene, and GAPDH terminator was gel purified and ligated in plasmid pAB24 which had been cut with BamHI. pAB24 is a yeast shuttle vector which contains the complete 2 μ sequences (Broach, In: Molecular Biology of the Yeast Saccharomyces, 1:445, Cold Spring Harbor Press (1981)) and pBR322 sequences. It also contains the yeast URA3 gene derived from plasmid YEp24 (Botstein et al, Gene (1979) 8:17) and the yeast $LEU2^d$ gene derived from plasmid pCl/l (described in European Patent Application publication no. EP0116201). Insertion of the expression cassette was in the BamHI site of pBR322, thus interrupting the gene for bacterial resistance to tetracycline. The resulting plasmid was designated pAB24F248 (or pAB24F251).

Plasmid pAB24F248 (or pAB24F251) was transformed into yeast strain S. cerevisiae 2150-2-3 Mata, adel, leu2-04, [$cir^{\bullet}$] (strain from the collection of L. Hartwell, University of Washington, Seattle) and plated on leu^- 8% glucose. Transformants were streaked on leu^- 8% glucose to obtain single colonies which were patched onto leu^- 8% glucose plates. Cells from 8 patches were grown for ~24 hr in leu^- 8% glucose minimal media and then inoculated (1:25 v/v) in YEP 1% glucose and grown for 96 hr at 30 °C. Samples (2.5 OD units) were collected for gel analysis at 24, 48, 72, and 96 hr.

Cell samples were boiled in SDS sample buffer (0.0675M Tris•Cl pH 6.8, 3% SDS, 10% glycerol) containing 50 mM DTT and run on 12.5% SDS-acrylamide gels. Coomassie staining of the gels showed a prominent band in the predicted 25.9 kd size range. This band accounted for ~20% of the total cell protein and was not found in pAB24 control extracts. It was seen in samples collected at each time point.

Western blot analyses were carried out on eight 48 hr samples of pAB24F248 and pAB24F251 transformants using rabbit anti-fibrolase sera. The antisera reacted strongly with the 25.9 kd band in all eight samples.

There were also lower molecular weight bands that reacted with the antisera, suggesting some degradation of the fibrolase in the yeast.

B. Construction of Yeast Expression Vector Encoding Corrected Native Fibrolase Sequence

Using pAB24F251 as a starting plasmid an internal expression plasmid was constructed which encodes the corrected native sequence (i.e., the authentic amino terminal sequence of mature southern copperhead fibrolase preceded by a Met residue [i.e., (Met) Gln-Gln-Arg-Phe-Pro-Gln-Arg....] and the amino acid changes at positions 123 and 170 (described supra) of the mature protein). Sequence changes were made by conventional in vitro mutagenesis techniques. This plasmid was designated pAB24F751.

S. cerevisiae cells transformed with pAB24F751 were observed to express a high level of protein corresponding in size to native fibrolase. Testing of cell lysates by the azocasein hydrolysis assay, however, showed no activity.

5. Construction of Plasmids For Secretory Expression of Mature Fibrolase and Profibrolase

A series of plasmids were made to direct the secretory expression of mature fibrolase or profibrolase (Profib). These plasmids are summarized in the following table.

Plasmid	Vector	Promoter	Leader	Fibrolase
pKS308	pAB24	GAP	αF	Mature Fibrolase
pKS311	pAB24	GAP	αF	Profib
pKS313	pAB24	ADH2/GAP	αF	Pro(KR)fib
pKS314	pAB24	GAP	αF	Pro(KR)fib
pKS316	pAB24	ADH2/GAP	αF(ΔKR)	Pro(KR)fib
pKS317	pAB24	GAP	αF(ΔKR)	Pro(KR)fib
pKS359	pAB38	GAP	αF	Profib
pKS362	pAB38	GAP	αF	Pro(KR)fib
pKS365	pAB38	GAP	αF(ΔKR)	Pro(KR)fib

GAP = glyceraldehyde-3-phosphate-dehydrogenase
ADH2 = alcohol degydrogenase 2
αF = S. cerevisiae alpha factor
KR = Lys-Arg processing site
ΔKR = minus Lys-Arg processing site

The fibrolase gene of pKS308 was derived from pAB24F751 and thus encodes the corrected native sequence. The profibrolase gene used in pKS311 was derived from Fib 51 and thus does not have the amino acid changes of the corrected native sequence but does include the 18 amino acid C-terminal extension of the Fib 51 sequence. A K. lactis counterpart of pKS311 (designated pKS359) was made using the K. lactis vector pAB38 instead of pAB24. pAB38 is a derivative of pUC18 (Yanisch-Perron et al., Gene (1985) 33:103) and was prepared as follows. pUC18 was cleaved with HindIII and a 1.2 kb HindIII fragment containing the URA3 gene (Botstein et al., Gene (1979) 8:17) was ligated into pUC18. Further, a PKDI (Falcone et al., Plasmid (1986) 15:248) EcoRI insert from Kluyveromyces drosophilarum was ligated into the EcoRI site of pUC18. Finally, thc vector was cleaved with NarI/HindIII (partial), filled in with Klenow, and religated to yield pAB38. The S. cerevisiae α-factor leader sequence and its incorporation into vectors is described in EPO Publication 116,201. The ADH2/GAP promoter and its incorporation into vectors is described in EPO Publication 164,556.

Expression of the pKS311 and pKS359 constructs in transformed S. cerevisiae and K. lactis yielded a substantial amount of immunoreactive species having the size of profibrolase. It thus appeared that a substantial portion of profibrolase was not being processed into the mature protein. Constructs were thus made in which a Lys-Arg processing site was introduced using synthetic oligonucleotides at the pro-mature junction which should be recognized by the yeast KEX2-encoded protease, the same enzyme that carries out the cleavage at the junction of the α-factor leader. Figure 7 shows the sequence generated by this mutagenesis process on a pAB24F751-derived α-factor profibrolase fusion construct. This sequence, being thus derived, includes the amino acid changes of the corrected native sequence but lacks the 18 C-terminal residues of the Fib 51 profibrolase sequence. The segment coding for those C-terminal residues may be introduced if desired. Such constructs were made using the GAP promoter (pKS314 in the table) and the ADH2/GAP promoter (pKS313 in the table). A corresponding construct was made based on the K. lactis vector pAB38 (pKS362 in the table).

pKS314 and pKS362 were introduced into S. cerevisiae and K. lactis strains and the production and secretion of mature fibrolase from the transformants was analyzed by SDS gel electrophoresis and Western blot analysis. Both secreted mostly mature fibrolase.

pKS314 and pKS362 each contain two Lys-Arg processing sites, one at the α-factor-pro junction and another at the pro-mature junction. In order to minimize the possibility of there being insufficient KEX2 protease to process all of the profibrolase being produced, a synthetic oligonucleotide adapter was used to remove the Lys-Arg site at the α-factor-pro junction. These constructs are designated pKS316, pKS317, and pKS365.

The fibrolase products produced using the expression vectors listed in the above table were tested for fibrolase activity by either the azocasein hydrolysis assay or the fluorometric assay.

The azocasein hydrolysis assay is carried out as follows. A solution of 2.5 g azocasein in 50ml 1% $NaHCO_3$ is prepared with stirring and warming to 60° C. The resulting solution is then dialyzed overnight

against 4 l of 1% $NaHCO_3$. A 50 µl portion of culture supernatent concentrated and dialyzed against 50 mM HEPES/pH 7.5 by ultrafiltration is then added to 1 ml of azocasein solution and the mixture is incubated for approximately 30 minutes-24 hours. One ml of 1.16 M perchloric acid is then added, the mixture is centrifuged for 10 minutes, and the absorbance of the supernatant is read at 390-440 nm against an appropriate control.

For the fluorometric assay, a fluorescein isothiocyanate casein (FITC-casein) assay reagent is prepared by mixing 1 part FITC-casein (5 mg/ml), 1 part 2X buffer (1X buffer = 50 mM HEPES-Na$^+$, 150 mM NaCl, 0.5 mM $ZnCl_2$, pH 7.5). Recombinant fibrolase product and snake venom fibrolase standards are diluted in 1X buffer containing 100 µg/ml BSA as a stabilizer.

Assay reagent, 80 µl, is brought to 37° and 20 µl of fibrolase sample or standard is added. After a 90 minutes incubation at 37°, the reaction is stopped by the addition of 300 µl of 7% TCA. After 30-60 minutes at 4°, the mixture is centrifuged for 6 minutes at 12,000 rpm. A 200 µl portion of the supernatant is removed and added to 1 ml of 0.5 M Tris-HCl, pH 8.0 and the fluorescence measured at an excitation wavelength of 490 nm and an emission wavelength of 525 nm, and the value compared to the fibrolase standards. For determination of non-fibrolase protolytic activity, assays were done in the presence of 10 mM EDTA, an inhibitor of fibrolase.

All of the recombinant fibrolase products produced using the expression vectors listed in the table exhibited fibrolase activity in these assays.

Modifications of the above described modes for carrying out the invention that are obvious to those of skill in the fields of enzyme chemistry, genetic engineering and/or medicine are intended to be within the scope of the following claims.

Claims

1. Recombinant DNA encoding a Crotalidus fibrolase.

2. The recombinant DNA of claim 1 wherein the fibrolase is southern copperhead fibrolase.

3. The recombinant DNA of claim 2 wherein the fibrolase comprises at least (a) amino acids 192-393 of the Fib 48 sequence shown in Figure 4, (b) amino acids 186-387 of the Fib 51 sequence of Figure 4 or (c) amino acids 185-387 of the Fib 51 sequence of Figure 4 with a Pro to Ser substitution at amino acid 307 of the Fib 51 sequence of Figure 4 and a Val to Met substitution at amino acid 354 of the Fib 51 sequence of Figure 4.

4. The recombinant DNA of claim 2 wherein the fibrolase is an analog of the fibrolase comprising at least (a) amino acids 192-393 of the Fib 48 sequence shown in Figure 4 (b) amino acids 186-387 of the Fib 51 sequence of Figure 4 or (c) amino acids 185-387 of the Fib 51 sequence of Figure 4 with a Pro to Ser substitution at amino acid 307 of the Fib 51 sequence of Figure 4 and a Val to Met substitution at amino acid 354 of the Fib 51 sequence of Figure 4.

5. An expression vector for expressing a Crotalidus fibrolase comprising the DNA of claim 1 and expression control sequences that are operably linked to said DNA and effective in directing expression of said DNA.

6. An expression vector for expressing southern copperhead fibrolase comprising the DNA of claim 2, 3 or 4 and expression control sequences that are operably linked to said DNA and effective in directing secretory expression of said DNA.

7. The expression vector of claim 6 wherein the expression vector is a yeast expression vector.

8. The expression vector of claim 7 wherein the expression control sequences include a yeast α-factor leader sequence for directing secretion of the fibrolase.

9. The expression vector of claim 8 wherein the DNA encodes the profibrolase sequence shown in Figure 7 and in which there is a Lys-Arg processing site at the pro-mature protein sequence.

10. A recombinant microorganism or cell containing the expression vector of claim 5 and being capable of producing a Crotalidus fibrolase.

11. A recombinant microorganism or cell containing the expression vector of claim 6 and being capable of producing southern copperhead fibrolase.

12. A recombinant yeast containing the expression vector of claims 7, 8 or 9.

13. A method of producing a Crotalidus fibrolase comprising growing the microorganism or cell of claim 10 under conditions which permit the expression of the fibrolase.

14. A method of producing southern copperhead fibrolase comprising growing the recombinant yeast cell of claim 12.

15. Recombinant homogeneous Crotalidus fibrolase free of snake proteins with which it is associated in venom.

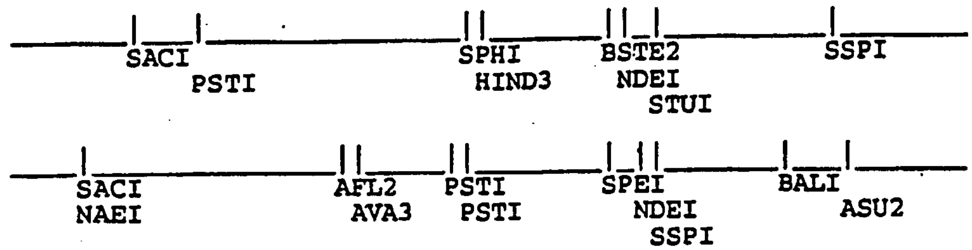
16. The recombinant Crotalidus fibrolase of claim 15 wherein the fibrolase is southern copperhead fibrolase.

17. The recombinant southern copperhead fibrolase of claim 16 comprising at least (a) amino acids 192-393 of the Fib 48 sequence shown in Figure 4, (b) amino acids 186-387 of the Fib 51 sequence of Figure 4 or (c) amino acids 185-387 of the Fib 51 sequence of Figure 4 with a Pro to Ser substitution at amino acid 307 of the Fib 51 sequence of Figure 4 and a Val to Met substitution at amino acid 354 of the Fib 51 sequence of Figure 4, or an analog thereof.

18. A pharmaceutical composition for treating a thromboembolic condition comprising the recombinant Crotalidus fibrolase of claim 15, 16 or 17 admixed with a pharmaceutically acceptable injectable carrier.

19. A method of treating a patient for a thromboembolic condition comprising administering a therapeutically effective amount of the recombinant Crotalidus fibrolase of claim 15, 16 or 17 to the patient.

FIB48 MAP

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  2  TCAGGTTGACTTGAAAGAAGGAAGAGATTGCCTGTCTTCCAGCCAAATCCAGCCTCCAAA
     AGTCCAACTGAACTTTCTTCCTTCTCTAACGGACAGAAGGTCGGTTTAGGTCGGAGGTTT

     MetIleGlnValLeuLeuValThrIleCysLeuThrAlaPheProTyrGlnGlySerSer
 62  ATGATCCAGGTTCTCTTGGTGACTATATGCTTAACAGCTTTTCCTTATCAAGGGAGCTCT
     TACTAGGTCCAAGAGAACCACTGATATACGAATTGTCGAAAAGGAATAGTTCCCTCGAGA

     115 SACI

     IleIleLeuGluSerGlyAsnValAsnAspTyrGluValValTyrProArgLysValThr
122  ATAATCCTGGAATCTGGGAACGTGAATGATTATGAAGTAGTGTATCCACGAAAAGTTACT
     TATTAGGACCTTAGACCCTTGCACTTACTAATACTTCATCACATAGGTGCTTTTCAATGA

     180 PSTI

     AlaValProArgGlyAlaValGlnProLysTyrGluAspAlaMetGlnTyrGluLeuLys
182  GCAGTGCCCAGAGGAGCAGTTCAGCCAAAGTATGAAGATGCCATGCAATATGAATTGAAA
     CGTCACGGGTCTCCTCGTCAAGTCGGTTTCATACTTCTACGGTACGTTATACTTAACTTT

     ValAsnGlyGluProValValLeuHisLeuGluLysAsnLysGlyLeuPheSerGluAsp
242  GTGAATGGAGAGCCAGTGGTCCTTCACCTGGAAAAAAATAAAGGACTTTTTTCAGAAGAT
     CACTTACCTCTCGGTCACCAGGAAGTGGACCTTTTTTTATTTCCTGAAAAAAGTCTTCTA

     TyrSerGluThrHisTyrSerProAspGlyArgGluIleThrThrTyrProLeuValGlu
302  TACAGCGAGACTCATTATTCCCCTGATGGCAGAGAAATTACAACATACCCCCTGGTTGAG
     ATGTCGCTCTGAGTAATAAGGGGACTACCGTCTCTTTAATGTTGTATGGGGGACCAACTC

     AspHisCysTyrTyrHisGlyArgIleGluAsnAspAlaAspSerThrAlaSerIleSer
362  GATCACTGCTATTATCATGGACGCATCGAGAATGATGCTGACTCAACTGCAAGCATCAGT
     CTAGTGACGATAATAGTACCTGCGTAGCTCTTACTACGACTGAGTTGACGTTCGTAGTCA

     AlaCysAsnGlyLeuLysGlyHisPheLysLeuGlnGlyGluMetTyrLeuIleGluPro
422  GCATGCAACGGTTTGAAAGGACATTTCAAGCTTCAAGGGGAGATGTACCTTATTGAACCA
     CGTACGTTGCCAAACTTTCCTGTAAAGTTCGAAGTTCCCCTCTACATGGAATAACTTGGT

     422 SPHI, 449 HIND3

     LeuGluLeuSerAspSerGluAlaHisAlaValTyrLysTyrGluAsnValGluLysGlu
482  TTGGAGCTTTCCGACAGTGAAGCCCATGCAGTCTACAAATATGAAAATGTAGAAAAAGAG
     AACCTCGAAAGGCTGTCACTTCGGGTACGTCAGATGTTTATACTTTTACATCTTTTTCTC
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FIG. 1-1

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     AspGluAlaProLysMetCysGlyValThrGlnAsnTrpGluSerTyrGluProIleLys
542  GATGAGGCCCCCAAAATGTGTGGGGTAACCCAGAATTGGGAATCATATGAGCCCATCAAA
     CTACTCCGGGGGTTTTACACACCCCATTGGGTCTTAACCCTTAGTATACTCGGGTAGTTT

     565 BSTE2, 585 NDEI

     LysAlaPheGlnLeuAsnLeuThrProGluGlnGlnGlyPheProGlnArgTyrValGlu
602  AAGGCCTTTCAGTTAAATCTTACTCCTGAACAACAAGGATTCCCCCAAAGATACGTTGAG
     TTCCGGAAAGTCAATTTAGAATGAGGACTTGTTGTTCCTAAGGGGGTTTCTATGCAACTC

     603 STUI

     LeuValIleValAlaAspHisArgMetTyrThrLysTyrAsnGlyAspSerAspLysIle
662  CTTGTCATAGTTGCGGATCACAGAATGTACACGAAATACAATGGTGATTCAGATAAGATA
     GAACAGTATCAACGCCTAGTGTCTTACATGTGCTTTATGTTACCACTAAGTCTATTCTAT

     ArgGlnTrpIleTyrArgMetValAsnThrIleAsnGluIleTyrArgProLeuAsnIle
722  AGACAATGGATATATCGAATGGTCAACACTATAAATGAGATTTACAGACCTTTGAATATT
     TCTGTTACCTATATAGCTTACCAGTTGTGATATTTACTCTAAATGTCTGGAAACTTATAA

     776 SSPI

     GlnPheValLeuValGlyLeuAspIleTrpSerLysLysAspLeuSerThrValThrSer
782  CAATTCGTACTGGTTGGCCTAGACATTTGGTCCAAGAAAGATTTGAGTACCGTGACATCA
     GTTAAGCATGACCAACCGGATCTGTAAACCAGGTTCTTTCTAAACTCATGGCACTGTAGT

     ValSerHisAspThrLeuAlaSerPheGluAsnTrpArgGlnThrAspLeuLeuAsnArg
842  GTATCACATGATACTTTGGCCTCATTTGAAAACTGGAGACAGACAGATTTGCTGAATCGC
     CATAGTGTACTATGAAACCGGAGTAAACTTTTGACCTCTGTCTGTCTAAACGACTTAGCG

     LysSerHisAspAsnAlaGlnLeuLeuThrAlaIleValPheAspGluGlyIleIleGly
902  AAAAGTCATGATAATGCCCAGTTACTCACGGCCATTGTCTTCGATGAAGGAATTATAGGA
     TTTTCAGTACTATTACGGGTCAATGAGTGCCGGTAACAGAAGCTACTTCCTTAATATCCT

     ArgAlaProLeuAlaGlyMetCysAspProMetPheSerValGlyIleValGluAspHis
962  AGAGCTCCCCTAGCCGGCATGTGTGACCCGATGTTTTCTGTAGGAATTGTTGAGGATCAT
     TCTCGAGGGGATCGGCCGTACACACTGGGCTACAAAAGACATCCTTAACAACTCCTAGTA

     963 SACI, 974 NAEI

     SerAlaIleAsnLeuLeuValAlaLeuThrMetAlaHisGluLeuGlyHisAsnLeuGly
1022 AGTGCAATAAATCTTTTGGTTGCACTTACAATGGCCCATGAGCTGGGTCATAATCTGGGC
     TCACGTTATTTAGAAAACCAACGTGAATGTTACCGGGTACTCGACCCAGTATTAGACCCG

     MetAspHisAspGlyAsnGlnCysHisCysGlyAlaAsnSerCysValMetAlaAspThr
1082 ATGGATCATGATGGAAATCAGTGTCATTGCGGTGCTAACTCGTGCGTTATGGCTGACACA
     TACCTAGTACTACCTTTAGTCACAGTAACGCCACGATTGAGCACGCAATACCGACTGTGT

     LeuSerAsnGlnProSerLysLeuPheSerAspCysSerLysLysTyrTyrGlnLysPhe
1142 CTAAGTAATCAACCTTCCAAACTATTCAGCGATTGTAGTAAGAAATACTATCAGAAGTTT
     GATTCATTAGTTGGAAGGTTTGATAAGTCGCTAACATCATTCTTTATGATAGTCTTCAAA

     LeuLysValLysAsnProGlnCysIleLeuAsnLysProLeuArgThrAspThrValSer
1202 CTTAAGGTTAAAAACCCACAATGCATTCTCAATAAACCCTTGAGAACAGATACTGTTTCA
     GAATTCCAATTTTTGGGTGTTACGTAAGAGTTATTTGGGAACTCTTGTCTATGACAAAGT

     1202 AFL2, 1222 AVA3

     ThrProValSerGlyAsnGluLeuLeuGluAlaOP
1262 ACTCCAGTTTCTGGAAATGAACTTTTGGAGGCGTGAGAAGAATGTGACTGTGGCTCTCCT
     TGAGGTCAAAGACCTTTACTTGAAAACCTCCGCACTCTTCTTACACTGACACCGAGAGGA

     1320 PSTI
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FIG. 1-2

1322 GCAGTCTGCAGCAACAGGCAGTGTGTTGATGTGACTACAGCCTAATAATCAACCTCTGGC
CGTCAGACGTCGTTGTCCGTCACACAACTACACTGATGTCGGATTATTAGTTGGAGACCG

1327 PSTI

1382 TTCTCTCAGATTTGATCTTGGAGATCCTTCTTTCAGAAGGTTTGGCTTCCCTGTAGTCCA
AAGAGAGTCTAAACTAGAACCTCTAGGAAGAAAGTCTTCCAAACCGAAGGGACATCAGGT

1442 AAGAGACCCATCTGCCTGCATCCTACTAGTAAATCACTCTTAGCTTTCATATGGAATCTA
TTCTCTGGGTAGACGGACGTAGGATGATCATTTAGTGAGAATCGAAAGTATACCTTAGAT

1466 SPEI, 1489 NDEI

1502 ACTTCTGCAATATTTCTTCTCCATATTTAATCTGTTTACCTTTTGCTGTAATCAAACCTT
TGAAGACGTTATAAAGAAGAGGTATAAATTAGACAAATGGAAAACGACATTAGTTTGGAA

1510 SSPI

1562 TTCCCACCACAAAGCTCTATGGGCATGTACAACACCAACGGCTTATCTGCTGTCAAGAAA
AAGGGTGGTGTTTCGAGATACCCGTACATGTTGTGGTTGCCGAATAGACGACAGTTCTTT

1622 AAAAATGGCCATTTTACCGTTTGCCAAAGCACATTTAATGCAACAAGTTCTGCCTTTTGA
TTTTTACCGGTAAAATGGCAAACGGTTTCGTGTAAATTACGTTGTTCAAGACGGAAAACT

1627 BALI

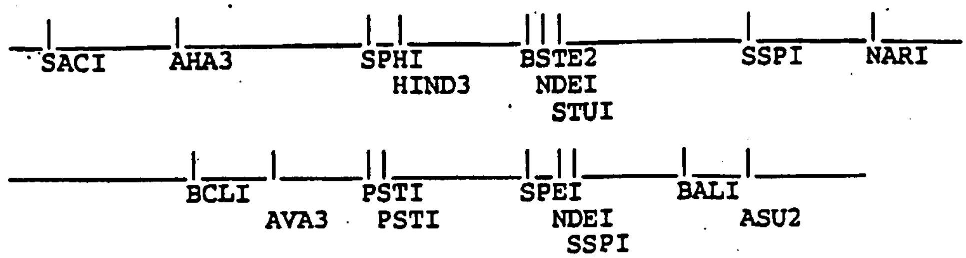
1682 GCTGGTGTATTCGAAGTGAATGTTTACTCTCCCAAAATTTCATGCTGGCTTTCACAAGAT
CGACCACATAAGCTTCACTTACAAATGAGAGGGTTTTAAAGTACGACCGAAAGTGTTCTA

1691 ASU2

1742 GTAGCTGCTTCCGTCAATAAACTAACTATTCTCATTCAAAAAAAA
CATCGACGAAGGCAGTTATTTGATTGATAAGAGTAAGTTTTTTTT

FIG. 1-3

FIB51 MAP

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    ValThrIleCysLeuAlaAlaPheProTyrGlnGlySerSerIleIleLeuGluSerGly
2   GTAACTATATGCTTAGCAGCTTTTCCTTATCAAGGGAGCTCTATAATCCTGGAATCTGGG
    CATTGATATACGAATCGTCGAAAAGGAATAGTTCCCTCGAGATATTAGGACCTTAGACCC

    37 SACI,

    AsnValAsnAspTyrGluValValTyrProArgLysValThrProValProArgGlyAla
62  AACGTTAATGATTATGAAGTAGTGTATCCACGAAAAGTCACTCCAGTGCCCAGAGGAGCA
    TTGCAATTACTAATACTTCATCACATAGGTGCTTTTCAGTGAGGTCACGGGTCTCCTCGT

    ValGlnProLysTyrGluAspAlaMetGlnTyrGluPheLysValAsnGlyGluProVal
122 GTTCAGCCAAAGTATGAAGATGCCATGCAATATGAATTTAAAGTGAATGGAGAGCCAGTG
    CAAGTCGGTTTCATACTTCTACGGTACGTTATACTTAAATTTCACTTACCTCTCGGTCAC

    158 AHA3,

    ValLeuHisLeuGluLysAsnLysGlyLeuPheSerGluAspTyrSerGluThrHisTyr
182 GTCCTTCACCTGGAAAAAAATAAAGGACTTTTTTCAGAAGATTACAGCGAGACTCATTAT
    CAGGAAGTGGACCTTTTTTTATTTCCTGAAAAAAGTCTTCTAATGTCGCTCTGAGTAATA

    SerProAspGlyArgGluIleThrThrTyrProLeuValGluAspHisCysTyrTyrHis
242 TCCCCTGATGGCAGAGAAATTACAACATACCCCCTGGTTGAGGATCACTGCTATTATCAT
    AGGGGACTACCGTCTCTTTAATGTTGTATGGGGGACCAACTCCTAGTGACGATAATAGTA

    GlyArgIleGluAsnAspAlaAspSerThrAlaSerIleSerAlaCysAsnGlyLeuLys
302 GGACGCATCGAGAATGATGCTGACTCAACTGCAAGCATCAGTGCATGCAACGGTTTGAAA
    CCTGCGTAGCTCTTACTACGACTGAGTTGACGTTCGTAGTCACGTACGTTGCCAAACTTT

    344 SPHI,

    GlyHisPheLysLeuGlnGlyGluMetTyrLeuIleGluProLeuGluLeuSerAspSer
362 GGACATTTCAAGCTTCAAGGGGAGATGTACCTTATTGAACCGTTGGAGCTTTCCGACAGT
    CCTGTAAAGTTCGAAGTTCCCCTCTACATGGAATAACTTGGCAACCTCGAAAGGCTGTCA

    371 HIND3,

    GluAlaHisAlaValTyrLysTyrGluAsnValGluLysGluAspGluAlaProLysMet
422 GAAGCCCATGCAGTCTACAAATATGAAAATGTAGAAAAAGAGGATGAGGCCCCCAAAATG
    CTTCGGGTACGTCAGATGTTTATACTTTTACATCTTTTTCTCCTACTCCGGGGGTTTTAC

    CysGlyValThrGlnAsnTrpGluSerTyrGluProIleLysLysAlaPheGlnLeuAsn
482 TGTGGGGTAACCCAGAATTGGGAATCATATGAGCCCATCAAAAAGGCCTTTCAGTTAAAT
    ACACCCCATTGGGTCTTAACCCTTAGTATACTCGGGTAGTTTTTCCGGAAAGTCAATTTA

    487 BSTE2, 507 NDEI, 525 STUI,
```

FIG. 2-1

FIG. 2-2

```
     LeuThrProGluGlnGlnArgPheProGlnArgTyrValGlnLeuValIleValAlaAsp
 542 CTTACTCCTGAACAACAAAGGTTCCCCCAAAGATATGTTCAGCTTGTCATAGTTGCAGAT
     GAATGAGGACTTGTTGTTTCCAAGGGGGTTTCTATACAAGTCGAACAGTATCAACGTCTA

     HisArgMetTyrMetLysTyrAsnAsnAspSerAsnLeuIleArgGlnTrpValHisGln
 602 CACAGAATGTACATGAAATACAATAATGATTCAAATTTGATAAGACAATGGGTACATCAA
     GTGTCTTACATGTACTTTATGTTATTACTAAGTTTAAACTATTCTGTTACCCATGTAGTT

     IleValAsnThrIleAsnGluIleTyrArgProLeuAsnIleGlnPheThrLeuValGly
 662 ATTGTCAACACTATAAATGAGATTTACAGACCTTTGAATATTCAATTCACACTGGTTGGC
     TAACAGTTGTGATATTTACTCTAAATGTCTGGAAACTTATAAGTTAAGTGTGACCAACCG

     698 SSPI,

     LeuGluIleTrpSerAsnGlnAspLeuIleThrValThrSerValSerHisAspThrLeu
 722 CTAGAAATTTGGTCCAACCAAGATTTGATTACCGTGACATCAGTATCACATGATACTTTG
     GATCTTTAAACCAGGTTGGTTCTAAACTAATGGCACTGTAGTCATAGTGTACTATGAAAC

     AlaSerPheGlyAsnTrpArgGluThrAspLeuLeuArgArgGlnArgHisAspAsnAla
 782 GCCTCATTTGGAAACTGGAGAGAGACAGACTTGCTAAGGCGCCAAAGACATGATAATGCC
     CGGAGTAAACCTTTGACCTCTCTCTGTCTGAACGATTCCGCGGTTTCTGTACTATTACGG

     819 NARI,

     GlnLeuLeuThrAlaIleAspPheAspGlyAspThrValGlyLeuAlaTyrValGlyGly
 842 CAGTTACTCACGGCCATTGACTTTGATGGAGACACTGTAGGATTGGCTTATGTGGGCGGT
     GTCAATGAGTGCCGGTAACTGAAACTACCTCTGTGACATCCTAACCGAATACACCCGCCA

     MetCysGlnLeuLysHisProThrGlyValIleGlnAspHisSerAlaIleAsnLeuLeu
 902 ATGTGCCAACTGAAGCATCCTACAGGAGTTATCCAGGATCATAGTGCAATAAATCTTTTG
     TACACGGTTGACTTCGTAGGATGTCCTCAATAGGTCCTAGTATCACGTTATTTAGAAAAC

     ValAlaLeuThrMetAlaHisGluLeuGlyHisAsnLeuGlyMetAsnHisAspGlyAsn
 962 GTTGCACTTACAATGGCCCATGAGCTGGGTCATAATCTGGGCATGAATCATGATGGAAAT
     CAACGTGAATGTTACCGGGTACTCGACCCAGTATTAGACCCGTACTTAGTACTACCTTTA

     GlnCysHisCysGlyAlaAsnSerCysValMetAlaAlaValLeuSerAspGlnProSer
1022 CAGTGTCATTGCGGTGCTAACTCGTGCGTCATGGCTGCTGTGCTAAGTGATCAACCCTCC
     GTCACAGTAACGCCACGATTGAGCACGCAGTACCGACGACACGATTCACTAGTTGGGAGG

     1069 BCLI,

     LysLeuPheSerAspCysSerLysLysAspTyrGlnThrPheLeuThrValAsnAsnPro
1082 AAACTATTCAGCGATTGTAGTAAGAAAGACTATCAGACGTTTCTTACGGTTAATAACCCA
     TTTGATAAGTCGCTAACATCATTCTTTCTGATAGTCTGCAAAGAATGCCAATTATTGGGT

     GlnCysIleLeuAsnLysProLeuArgThrAspThrValSerThrProValSerGlyAsn
1142 CAATGCATTCTCAATAAACCCTTGAGAACAGATACTGTTTCAACTCCAGTTTCTGGAAAT
     GTTACGTAAGAGTTATTTGGGAACTCTTGTCTATGACAAAGTTGAGGTCAAAGACCTTTA

     1144 AVA3,

     GluLeuLeuGluAlaOP
1202 GAACTTTTGGAGGCGTGAGAAGAATGTGACTGTGGCTCTCCTGCAGTCTGCAGCAACAGG
     CTTGAAAACCTCCGCACTCTTCTTACACTGACACCGAGAGGACGTCAGACGTCGTTGTCC

     1242 PSTI, 1249 PSTI,

1262 CAGTGTGTTGATGTGACTACAGCCTAATAATCAACCTCTGGCTTCTCTCAGATTTGATCT
     GTCACACAACTACACTGATGTCGGATTATTAGTTGGAGACCGAAGAGAGTCTAAACTAGA

1322 TGGAGATCCTTCTTTCAGGAGGTTTGGCTTCCCTGTAGTCCAAAGAGACCCATCTGCCTG
     ACCTCTAGGAAGAAAGTCCTCCAAACCGAAGGGACATCAGGTTTCTCTGGGTAGACGGAC
```

1382 CATCCTACTAGTAAATCACTCTTAGCTTTCATATGGAATCTAACTTCTGCAATATTTCTT
GTAGGATGATCATTTAGTGAGAATCGAAAGTATACCTTAGATTGAAGACGTTATAAAGAA

1388 SPEI, 1411 NDEI, 1432 SSPI,

1442 CTCCATATTTAATCTGTAATCAAACCTTTTCCCACCACAAAGCTCTATGTGCATGTACAA
GAGGTATAAATTAGACATTAGTTTGGAAAAGGGTGGTGTTTCGAGATACACGTACATGTT

1502 CACCAACGGCTTATCTGCTGTCAAGAAAAAAAATGGCCATTTTACCGTTTGCCAAAGCAC
GTGGTTGCCGAATAGACGACAGTTCTTTTTTTTACCGGTAAAATGGCAAACGGTTTCGTG

1535 BALI,

1562 ATTTAATGCAACAAGTTCTGCCTTTTGAGCTGGTGTATTCGAAGTGAATGTTTACTCTCC
TAAATTACGTTGTTCAAGACGGAAAACTCGACCACATAAGCTTCACTTACAAATGAGAGG

1599 ASU2,

1622 CAAAATTTCATGCTGGCTTTCCAAGATGTAGCTGCTTCCGTCAATAAACTAACTATTCTC
GTTTTAAAGTACGACCGAAAGGTTCTACATCGACGAAGGCAGTTATTTGATTGATAAGAG

1682 ATTAAAAAAAAAAAA
TAATTTTTTTTTTTT

FIG. 2-3

FIG. 3-1

```
               70        80        90       100       110       120
Fib48   AATGATCCAGGTTCTCTTGGTGACTATATGCTTAACAGCTTTTCCTTATCAAGGGAGCTC
                            *            *
Fib51                       GGTAAGTATATGCTTAGCAGCTTTTCCTTATCAAGGGAGCTC
                                    10        20        30        40

              130       140       150       160       170       180
Fib48   TATAATCCTGGAATCTGGGAACGTGAATGATTATGAAGTAGTGTATCCACGAAAAGTTAC
                                *                                *
Fib51   TATAATCCTGGAATCTGGGAACGTTAATGATTATGAAGTAGTGTATCCACGAAAAGTCAC
              50        60        70        80        90       100

              190       200       210       220       230       240
Fib48   TGCAGTGCCCAGAGGAGCAGTTCAGCCAAAGTATGAAGATGCCATGCAATATGAATTGAA
         *                                                        *
Fib51   TCCAGTGCCCAGAGGAGCAGTTCAGCCAAAGTATGAAGATGCCATGCAATATGAATTTAA
             110       120       130       140       150       160

              250       260       270       280       290       300
Fib48   AGTGAATGGAGAGCCAGTGGTCCTTCACCTGGAAAAAAATAAAGGACTTTTTTCAGAAGA

Fib51   AGTGAATGGAGAGCCAGTGGTCCTTCACCTGGAAAAAAATAAAGGACTTTTTTCAGAAGA
             170       180       190       200       210       220

              310       320       330       340       350       360
Fib48   TTACAGCGAGACTCATTATTCCCCTGATGGCAGAGAAATTACAACATACCCCCTGGTTGA

Fib51   TTACAGCGAGACTCATTATTCCCCTGATGGCAGAGAAATTACAACATACCCCCTGGTTGA
             230       240       250       260       270       280

              370       380       390       400       410       420
Fib48   GGATCACTGCTATTATCATGGACGCATCGAGAATGATGCTGACTCAACTGCAAGCATCAG

Fib51   GGATCACTGCTATTATCATGGACGCATCGAGAATGATGCTGACTCAACTGCAAGCATCAG
             290       300       310       320       330       340

              430       440       450       460       470       480
Fib48   TGCATGCAACGGTTTGAAAGGACATTTCAAGCTTCAAGGGGAGATGTACCTTATTGAACC

Fib51   TGCATGCAACGGTTTGAAAGGACATTTCAAGCTTCAAGGGGAGATGTACCTTATTGAACC
             350       360       370       380       390       400

              490       500       510       520       530       540
Fib48   ATTGGAGCTTTCCGACAGTGAAGCCCATGCAGTCTACAAATATGAAAATGTAGAAAAAGA

Fib51   GTTGGAGCTTTCCGACAGTGAAGCCCATGCAGTCTACAAATATGAAAATGTAGAAAAAGA
             410       420       430       440       450       460

              550       560       570       580       590       600
Fib48   GGATGAGGCCCCCAAAATGTGTGGGGTAACCCAGAATTGGGAATCATATGAGCCCATCAA

Fib51   GGATGAGGCCCCCAAAATGTGTGGGGTAACCCAGAATTGGGAATCATATGAGCCCATCAA
             470       480       490       500       510       520

              610       620       630       640       650       660
Fib48   AAAGGCCTTTCAGTTAAATCTTACTCCTGAACAACAAGGATTCCCCCAAAGATACGTTGA
                                                 * *              *  *
Fib51   AAAGGCCTTTCAGTTAAATCTTACTCCTGAACAACAAAGGTTCCCCCAAAGATATGTTCA
             530       540       550       560       570       580

              670       680       690       700       710       720
Fib48   GCTTGTCATAGTTGCGGATCACAGAATGTACACGAAATACAATGGTGATTCAGATAAGAT
                       *                *          **       *  **
Fib51   GCTTGTCATAGTTGCAGATCACAGAATGTACATGAAATACAATAATGATTCAAATTTGAT
             590       600       610       620       630       640
```

FIG. 3-2

```
              730       740       750       760       770       780
Fib48  AAGACAATGGATATATCGAATGGTCAACACTATAAATGAGATTTACAGACCTTTGAATAT
                 *  *   *   *
Fib51  AAGACAATGGGTACATCAAATTGTCAACACTATAAATGAGATTTACAGACCTTTGAATAT
            650       660       670       680       690       700

              790       800       810       820       830       840
Fib48  TCAATTCGTACTGGTTGGCCTAGACATTTGGTCCAAGAAAGATTTGAGTACCGTGACATC
              **               *           **         *
Fib51  TCAATTCACACTGGTTGGCCTAGAAATTTGGTCCAACCAAGATTTGATTACCGTGACATC
            710       720       730       740       750       760

              850       860       870       880       890       900
Fib48  AGTATCACATGATACTTTGGCCTCATTTGAAAACTGGAGACAGACAGATTTGCTGAATCG
                                    *          *       *     * **
Fib51  AGTATCACATGATACTTTGGCCTCATTTGGAAACTGGAGAGAGACAGACTTGCTAAGGCG
            770       780       790       800       810       820

              910       920       930       940       950       960
Fib48  CAAAAGTCATGATAATGCCCAGTTACTCACGGCCATTGTCTTCGATGAAGGAATTATAGG
        *    *                               *   *    *  ** * *
Fib51  CCAAAGACATGATAATGCCCAGTTACTCACGGCCATTGACTTTGATGGAGACACTGTAGG
            830       840       850       860       870       880

              970       980       990      1000      1010      1020
Fib48  AAGAGCTCCCCTAGCCGGCATGTGTGACCCGATGTTTTCTGTAGGAATTGTTGAGGATCA
        ***   **** * *   *     ** * *  * ** *  **    *  * **
Fib51  ATTGGCTTATGTGGGCGGTATGTGCCAACTGAAGCATCCTACAGGAGTTATCCAGGATCA
            890       900       910       920       930       940

             1030      1040      1050      1060      1070      1080
Fib48  TAGTGCAATAAATCTTTTGGTTGCACTTACAATGGCCCATGAGCTGGGTCATAATCTGGG

Fib51  TAGTGCAATAAATCTTTTGGTTGCACTTACAATGGCCCATGAGCTGGGTCATAATCTGGG
            950       960       970       980       990      1000

             1090      1100      1110      1120      1130      1140
Fib48  CATGGATCATGATGGAAATCAGTGTCATTGCGGTGCTAACTCGTGCGTTATGGCTGACAC
           *                                           *       ****
Fib51  CATGAATCATGATGGAAATCAGTGTCATTGCGGTGCTAACTCGTGCGTCATGGCTGCTGT
           1010      1020      1030      1040      1050      1060

             1150      1160      1170      1180      1190      1200
Fib48  ACTAAGTAATCAACCTTCCAAACTATTCAGCGATTGTAGTAAGAAATACTATCAGAAGTT
       *      *       *                              *         *
Fib51  GCTAAGTGATCAACCCTCCAAACTATTCAGCGATTGTAGTAAGAAAGACTATCAGACGTT
           1070      1080      1090      1100      1110      1120

             1210      1220      1230      1240      1250      1260
Fib48  TCTTAAGGTTAAAAACCCACAATGCATTCTCAATAAACCCTTGAGAACAGATACTGTTTC
            *      *
Fib51  TCTTACGGTTAATAACCCACAATGCATTCTCAATAAACCCTTGAGAACAGATACTGTTTC
           1130      1140      1150      1160      1170      1180

             1270      1280      1290      1300      1310      1320
Fib48  AACTCCAGTTTCTGGAAATGAACTTTTGGAGGCGTGAGAAGAATGTGACTGTGGCTCTCC

Fib51  AACTCCAGTTTCTGGAAATGAACTTTTGGAGGCGTGAGAAGAATGTGACTGTGGCTCTCC
           1190      1200      1210      1220      1230      1240

             1330      1340      1350      1360      1370      1380
Fib48  TGCAGTCTGCAGCAACAGGCAGTGTGTTGATGTGACTACAGCCTAATAATCAACCTCTGG

Fib51  TGCAGTCTGCAGCAACAGGCAGTGTGTTGATGTGACTACAGCCTAATAATCAACCTCTGG
           1250      1260      1270      1280      1290      1300
```

```
                 1390      1400      1410      1420      1430      1440
Fib48      CTTCTCTCAGATTTGATCTTGGAGATCCTTCTTTCAGAAGGTTTGGCTTCCCTGTAGTCC
                                                *
Fib51      CTTCTCTCAGATTTGATCTTGGAGATCCTTCTTTCAGGAGGTTTGGCTTCCCTGTAGTCC
               1310      1320      1330      1340      1350      1360

                 1450      1460      1470      1480      1490      1500
Fib48      AAAGAGACCCATCTGCCTGCATCCTACTAGTAAATCACTCTTAGCTTTCATATGGAATCT

Fib51      AAAGAGACCCATCTGCCTGCATCCTACTAGTAAATCACTCTTAGCTTTCATATGGAATCT
               1370      1380      1390      1400      1410      1420

                 1510      1520      1530      1540      1550      1560
Fib48      AACTTCTGCAATATTTCTTCTCCATATTTAATCTGTTTACCTTTTGCTGTAATCAAACCT
                                            **************
Fib51      AACTTCTGCAATATTTCTTCTCCATATTTAATC--------------TGTAATCAAACCT
               1430      1440      1450                    1460

                 1570      1580      1590      1600      1610      1620
Fib48      TTTCCCACCACAAAGCTCTATGGGCATGTACAACACCAACGGCTTATCTGCTGTCAAGAA
                                 *
Fib51      TTTCCCACCACAAAGCTCTATGTGCATGTACAACACCAACGGCTTATCTGCTGTCAAGAA
         1470      1480      1490      1500      1510      1520

                 1630      1640      1650      1660      1670      1680
Fib48      AAAAAATGGCCATTTTACCGTTTGCCAAAGCACATTTAATGCAACAAGTTCTGCCTTTTG

Fib51      AAAAAATGGCCATTTTACCGTTTGCCAAAGCACATTTAATGCAACAAGTTCTGCCTTTTG
         1530      1540      1550      1560      1570      1580

                 1690      1700      1710      1720      1730      1740
Fib48      AGCTGGTGTATTCGAAGTGAATGTTTACTCTCCCAAAATTTCATGCTGGCTTTCACAAGA
                                                                 *
Fib51      AGCTGGTGTATTCGAAGTGAATGTTTACTCTCCCAAAATTTCATGCTGGCTTTC-CAAGA
         1590      1600      1610      1620      1630      1640

                 1750      1760      1770      1780
Fib48      TGTAGCTGCTTCCGTCAATAAACTAACTATTCTCATTCAAAAAAAA
                                                *
Fib51      TGTAGCTGCTTCCGTCAATAAACTAACTATTCTCATTAAAAAAAAAAAAA
          1650      1660      1670      1680      1690
```

94.3% identity in 1708 bp overlap

FIG. 3-3

```
                   10        20        30        40        50        60
Fib48      MIQVLLVTICLTAFPYQGSSIILESGNVNDYEVVYPRKVTAVPRGAVQPKYEDAMQYELK
                      *                            *                 *
Fib51            VTICLAAFPYQGSSIILESGNVNDYEVVYPRKVTPVPRGAVQPKYEDAMQYEFK
                           10        20        30        40        50

                   70        80        90       100       110       120
Fib48      VNGEPVVLHLEKNKGLFSEDYSETHYSPDGREITTYPLVEDHCYYHGRIENDADSTASIS

Fib51      VNGEPVVLHLEKNKGLFSEDYSETHYSPDGREITTYPLVEDHCYYHGRIENDADSTASIS
               60        70        80        90       100       110

                  130       140       150       160       170       180
Fib48      ACNGLKGHFKLQGEMYLIEPLELSDSEAHAVYKYENVEKEDEAPKMCGVTQNWESYEPIK

Fib51      ACNGLKGHFKLQGEMYLIEPLELSDSEAHAVYKYENVEKEDEAPKMCGVTQNWESYEPIK
              120       130       140       150       160       170

                  190       200       210       220       230       240
Fib48      KAFQLNLTPEQQGFPQRYVELVIVADHRMYTKYNGDSDKIRQWIYRMVNTINEIYRPLNI
                       *      *          *   *  **    ****
Fib51      KAFQLNLTPEQQRFPQRYVQLVIVADHRMYMKYNNDSNLIRQWVHQIVNTINEIYRPLNI
              180       190       200       210       220       230

                  250       260       270       280       290       300
Fib48      QFVLVGLDIWSKKDLSTVTSVSHDTLASFENWRQTDLLNRKSHDNAQLLTAIVFDEGIIG
             *    *   **  *             *   *    * **          *  ****
Fib51      QFTLVGLEIWSNQDLITVTSVSHDTLASFGNWRETDLLRRQRHDNAQLLTAIDFDGDTVG
              240       250       260       270       280       290

                  310       320       330       340       350       360
Fib48      RAPLAGMCDPMFSVGIVEDHSAINLLVALTMAHELGHNLGMDHDGNQCHCGANSCVMADT
             ***   ****** ***                       *                **
Fib51      LAYVGGMCQLKHPTGVIQDHSAINLLVALTMAHELGHNLGMNHDGNQCHCGANSCVMAAV
              300       310       320       330       340       350

                  370       380       390       400       410
Fib48      LSNQPSKLFSDCSKKYYQKFLKVKNPQCILNKPLRTDTVSTPVSGNELLEA
             *            *  *  * *
Fib51      LSDQPSKLFSDCSKKDYQTFLTVNNPQCILNKPLRTDTVSTPVSGNELLEA
              360       370       380       390       400
```

87.9 % identity in 405 residue overlap

FIG. 4

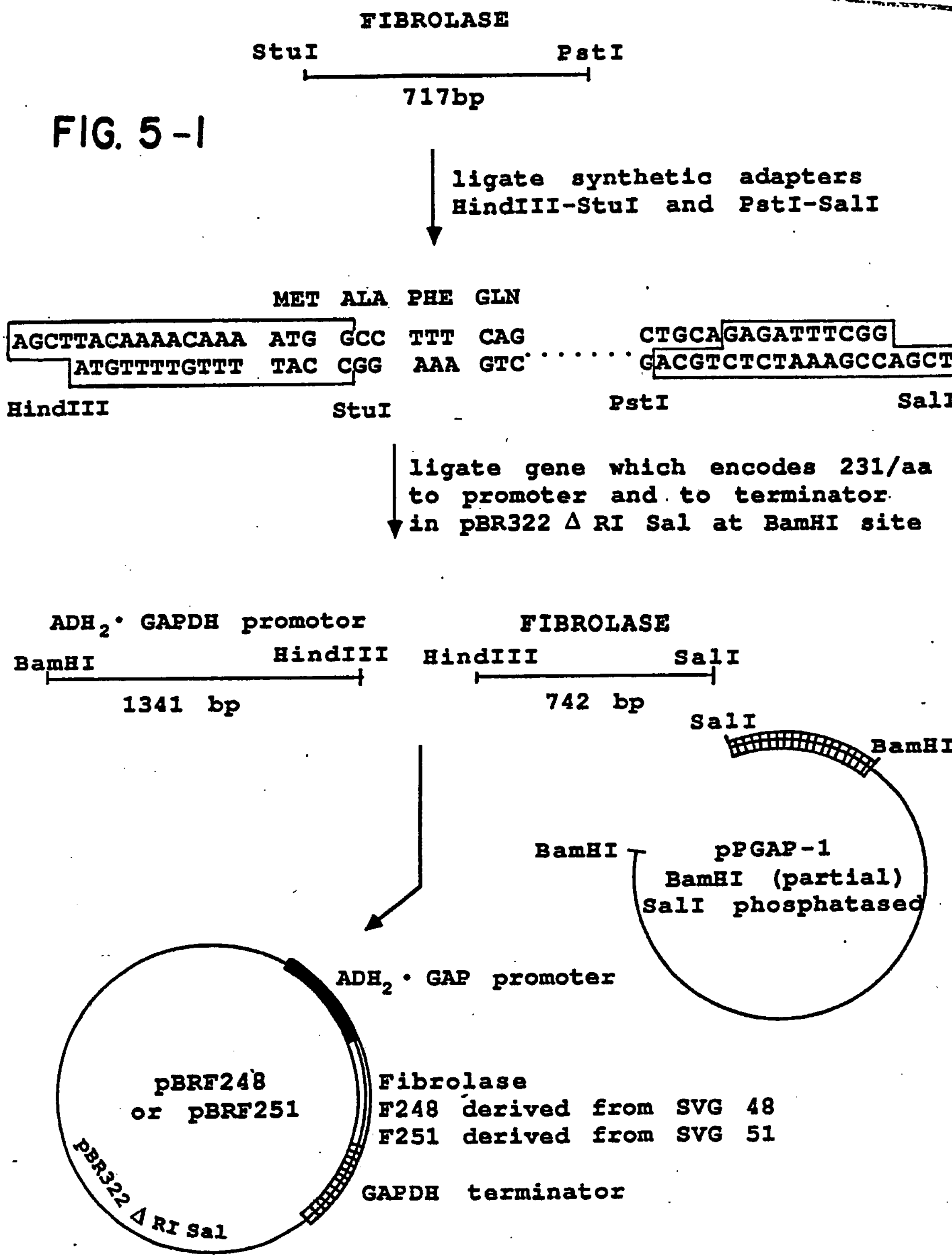
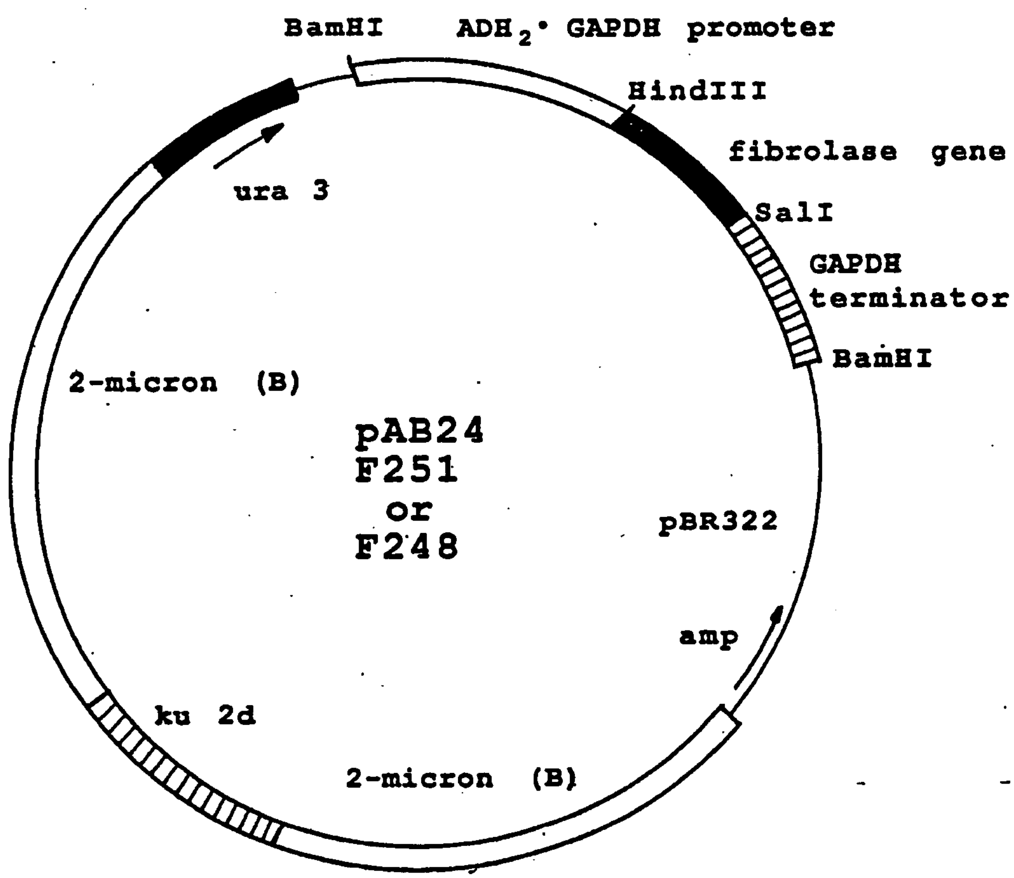

FIBROLASE
StuI
PstI
717bp
ligate synthetic adapters
HindIII-StuI and PstI-SalI
MET ALA PHE GLN
AGCTTACAAAACAAA ATG GCC TTT CAG
ATGTTTTGTTT TAC CGG AAA GTC
CTGCAGAGATTTCGG
GACGTCTCTAAAGCCAGCT
HindIII
StuI
PstI
SalI
ligate gene which encodes 231/aa
to promoter and to terminator
in pBR322 Δ RI Sal at BamHI site
ADH2 • GAPDH promotor
BamHI
HindIII
1341 bp
FIBROLASE
HindIII
SalI
742 bp
SalI
BamHI
BamHI
pPGAP-1
BamHI (partial)
SalI phosphatased
ADH2 • GAP promoter
pBRF248
or pBRF251
Fibrolase
F248 derived from SVG 48
F251 derived from SVG 51
GAPDH terminator
pBR322 Δ RI Sal

FIG. 5-1

FIG. 5-2

gel isolate BamHI expression cassette and ligate into pAB24



1 GATCCTTCAATATGCGCACATACGCTGTTATGTTCAAGGTCCCTTCGTTTAAGAACGAAA
CTAGGAAGTTATACGCGTGTATGCGACAATACAAGTTCCAGGGAAGCAAATTCTTGCTTT

13 FSPI MSTI,

61 GCGGTCTTCCTTTTGAGGGATGTTTCAAGTTGTTCAAATCTATCAAATTTGCAAATCCCC
CGCCAGAAGGAAAACTCCCTACAAAGTTCAACAAGTTTAGATAGTTTAAACGTTTAGGGG

121 AGTCTGTATCTAGAGCGTTGAATCGGTGATGCGATTTGTTAATTAAATTGATGGTGTCAC
TCAGACATAGATCTCGCAACTTAGCCACTACGCTAAACAATTAATTTAACTACCACAGTG

129 XBAI,

181 CATTACCAGGTCTAGATATACCAATGGCAAACTGAGCACAACAATACCAGTCCGGATCAA
GTAATGGTCCAGATCTATATGGTTACCGTTTGACTCGTGTTGTTATGGTCAGGCCTAGTT

191 XBAI,

241 CTGGCACCATCTCTCCCGTAGTCTCATCTAATTTTTCTTCCGGATGAGGTTCCAGATATA
GACCGTGGTAGAGAGGGCATCAGAGTAGATTAAAAAGAAGGCCTACTCCAAGGTCTATAT

301 CCGCAACACCTTTATTATGGTTTCCCTGAGGGAATAATAGAATGTCCCATTCGAAATCAC
GGCGTTGTGGAAATAATACCAAAGGGACTCCCTTATTATCTTACAGGGTAAGCTTTAGTG

325 MST2, 350 ASU2,

361 CAATTCTAAACCTGGGCGAATTGTATTTCGGGTTTGTTAACTCGTTCCAGTCAGGAATGT
GTTAAGATTTGGACCCGCTTAACATAAAGCCCAAACAATTGAGCAAGGTCAGTCCTTACA

396 HPAI,

421 TCCACGTGAAGCTATCTTCCAGCAAAGTCTCCACTTCTTCATCAAATTGTGGAGAATACT
AGGTGCACTTCGATAGAAGGTCGTTTCAGAGGTGAAGAAGTAGTTTAACACCTCTTATGA

481 CCCAATGCTCTTATCTATGGGACTTCCGGGAAACACAGTACCGATACTTCCCAATTCGTC
GGGTTACGAGAATAGATACCCTGAAGGCCCTTTGTGTCATGGCTATGAAGGGTTAAGCAG

541 TTCAGAGCTCATTGTTTGTTTGAAGAGACTAATCAAAGAATCGTTTTCTCAAAAAAATTA
AAGTCTCGAGTAACAAACAAACTTCTCTGATTAGTTTCTTAGCAAAAGAGTTTTTTTAAT

545 SACI,

601 ATATCTTAACTGATAGTTTGATCAAAGGGGCAAAACGTAGGGGCAAACAAACGGAAAAAT
TATAGAATTGACTATCAAACTAGTTTCCCCGTTTTGCATCCCCGTTTGTTTGCCTTTTTA

619 BCLI,

661 CGTTTCTCAAATTTTCTGATGCCAAGAACTCTAACCAGTCTTATCTAAAAATTGCCTTAT

FIG. 6-1

```
     GCAAAGAGTTTAAAAGACTACGGTTCTTGAGATTGGTCAGAATAGATTTTTAACGGAATA

721  GATCCGTCTCTCCGGTTACAGCCTGTGTAACTGATTAATCCTGCCTTTCTAATCACCATT
     CTAGGCAGAGAGGCCAATGTCGGACACATTGACTAATTAGGACGGAAAGATTAGTGGTAA

781  CTAATGTTTTAATTAAGGGATTTTGTCTTCATTAACGGCTTTCGCTCATAAAAATGTTAT
     GATTACAAAATTAATTCCCTAAAACAGAAGTAATTGCCGAAAGCGAGTATTTTTACAATA

841  GACGTTTTGCCCGCAGGCGGGAAACCATCCACTTCACGAGACTGATCTCCTCTGCCGGAA
     CTGCAAAACGGGCGTCCGCCCTTTGGTAGGTGAAGTGCTCTGACTAGAGGAGACGGCCTT

901  CACCGGGCATCTCCAACTTATAAGTTGGAGAAATAAGAGAATTTCAGATTGAGAGAATGA
     GTGGCCCGTAGAGGTTGAATATTCAACCTCTTTATTCTCTTAAAGTCTAACTCTCTTACT

961  AAAAAAAAAACCCTTAGTTCATAGGTCCATTCTCTTAGCGCAACTACAGAGAACAGGGGC
     TTTTTTTTTTGGGAATCAAGTATCCAGGTAAGAGAATCGCGTTGATGTCTCTTGTCCCCG

1021 ACAAACAGGCAAAAAACGGGCACAACCTCAATGGAGTGATGCAACCTGCCTGGAGTAAAT
     TGTTTGTCCGTTTTTTGCCCGTGTTGGAGTTACCTCACTACGTTGGACGGACCTCATTTA

1081 GATGACACAAGGCAATTGACCCACGCATGTATCTATCTCATTTTCTTACACCTTCTATTA
     CTACTGTGTTCCGTTAACTGGGTGCGTACATAGATAGAGTAAAAGAATGTGGAAGATAAT

1141 CCTTCTGCTCTCTCTGATTTGGAAAAAGCTGAAAAAAAAGGTTGAAACCAGTTCCCTGAA
     GGAAGACGAGAGAGACTAAACCTTTTTCGACTTTTTTTTCCAACTTTGGTCAAGGGACTT

     1198 XMNI,

1201 ATTATTCCCCTACTTGACTAATAAGTATATAAAGACGGTAGGTATTGATTGTAATTCTGT
     TAATAAGGGGATGAACTGATTATTCATATATTTCTGCCATCCATAACTAACATTAAGACA

1261 AAATCTATTTCTTAAACTTCTTAAATTCTACTTTTATAGTTAGTCTTTTTTTTAGTTTTA
     TTTAGATAAAGAATTTGAAGAATTTAAGATGAAAATATCAATCAGAAAAAAAATCAAAAT

     1317 AHA3,

1321 AAACACCAAGAACTTAGTTTCGAATAAACACACATAAACAAACAAGCTT
     TTTGTGGTTCTTGAATCAAAGCTTATTTGTGTGTATTTGTTTGTTCGAA

     1339 ASU2, 1364 HIND3,
```

FIG. 6-2

FIG. 7-1 INSERTION OF A KEX2 PROCESSING SITE INTO α-FACTOR/PROFIBROLASE

```
1                                   10                                                          20          ***                          30
Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala Ser Ser Ala Leu Ala Ala Pro Val Asn Thr Thr Thr Glu Asp Glu Thr
ATG AGA TTT CCT TCA ATT TTT ACT GCA GTT TTA TTC GCA GCA TCC TCC GCA TTA GCT GCT CCA GTC AAC ACT ACA ACA GAA GAT GAA ACG

                                    40                                                          50                      ***          60
Ala Gln Ile Pro Ala Glu Ala Val Ile Gly Tyr Leu Asp Leu Glu Gly Asp Phe Asp Val Ala Val Leu Pro Phe Ser Asn Ser Thr Asn
GCA CAA ATT CCG GCT GAA GCT GTC ATC GGT TAC TTA GAT TTA GAA GGG GAT TTC GAT GTT GCT GTT TTG CCA TTT TCC AAC AGC ACA AAT

                        ***         70                                                          80                                   90
Asn Gly Leu Leu Phe Ile Asn Thr Thr Ile Ala Ser Ile Ala Ala Lys Glu Glu Gly Val Ser Leu Asp Lys Arg Ser Ser Ile Ile Leu
AAC GGG TTA TTG TTT ATA AAT ACT ACT ATT GCC AGC ATT GCT GCT AAA GAA GAA GGG GTA TCT CTA GAT AAA AGG AGC TCT ATA ATC CTG

                                    100                                                         110                                  120
Glu Ser Gly Asn Val Asn Asp Tyr Glu Val Val Tyr Pro Arg Lys Val Thr Pro Val Pro Arg Gly Ala Val Gln Pro Lys Tyr Glu Asp
GAA TCT GGG AAC GTT AAT GAT TAT GAA GTA GTG TAT CCA CGA AAA GTC ACT CCA GTG CCC AGA GGA GCA GTT CAG CCA AAG TAT GAA GAT

                                    130                                                         140                                  150
Ala Met Gln Tyr Glu Phe Lys Val Asn Gly Glu Pro Val Val Leu His Leu Glu Lys Asn Lys Gly Leu Phe Ser Glu Asp Tyr Ser Glu
GCC ATG CAA TAT GAA TTT AAA GTG AAT GGA GAG CCA GTG GTC CTT CAC CTG GAA AAA AAT AAA GGA CTT TTT TCA GAA GAT TAC AGC GAG

                                    160                                                         170                                  180
Thr His Tyr Ser Pro Asp Gly Arg Glu Ile Thr Thr Tyr Pro Leu Val Glu Asp His Cys Tyr Tyr His Gly Arg Ile Glu Asn Asp Ala
ACT CAT TAT TCC CCT GAT GGC AGA GAA ATT ACA ACA TAC CCC CTG GTT GAG GAT CAC TGC TAT TAT CAT GGA CGC ATC GAG AAT GAT GCT

                                    190                                                         200                                  210
Asp Ser Thr Ala Ser Ile Ser Ala Cys Asn Gly Leu Lys Gly His Phe Lys Leu Gln Gly Glu Met Tyr Leu Ile Glu Pro Leu Glu Leu
GAC TCA ACT GCA AGC ATC AGT GCA TGC AAC GGT TTG AAA GGA CAT TTC AAG CTT CAA GGG GAG ATG TAC CTT ATT GAA CCG TTG GAG CTT

                                    220                                                         230                                  240
Ser Asp Ser Glu Ala His Ala Val Tyr Lys Tyr Glu Asn Val Glu Lys Glu Asp Glu Ala Pro Lys Met Cys Gly Val Thr Gln Asn Trp
TCC GAC AGT GAA GCC CAT GCA GTC TAC AAA TAT GAA AAT GTA GAA AAA GAG GAT GAG GCC CCC AAA ATG TGT GGG GTA ACC CAG AAT TGG
```

FIG. 7-2

```
                                                            LYS ARG
                                    250         ***                         260                                     270
Glu Ser Tyr Glu Pro Ile Lys Lys Ala Phe Gln Leu Asn Leu Thr Pro Glu Gln Gln Arg Phe Pro Gln Arg Tyr Val Gln Leu Val Ile
GAA TCA TAT GAG CCC ATC AAA AAG GCC TTT CAG TTA AAT CTT ACT CCT GAA CAA CAA AGG TTC CCC CAA AGA TAT GTT CAG CTT GTC ATA

                                            Gly
                        Asn Thr     280     ***         Asp Lys             290                                     300
Val Ala Asp His Arg Met Tyr Met Lys Tyr Asn Asn Asp Ser Asn Leu Ile Arg Gln Trp Val His Gln Ile Val Asn Thr Ile Asn Glu
GTT GCA GAT CAC AGA ATG TAC ATG AAA TAC AAT AAT GAT TCA AAT TTG ATA AGA CAA TGG GTA CAT CAA ATT GTC AAC ACT ATA AAT GAG

                                    310                                     320                                     330
Ile Tyr Arg Pro Leu Asn Ile Gln Phe Thr Leu Val Gly Leu Glu Ile Trp Ser Asn Gln Asp Leu Ile Thr Val Thr Ser Val Ser His
ATT TAC AGA CCT TTG AAT ATT CAA TTC ACA CTG GTT GGC CTA GAA ATT TGG TCC AAC CAA GAT TTG ATT ACC GTG ACA TCA GTA TCA CAT

                                    340                                     350                                     360
Asp Thr Leu Ala Ser Phe Gly Asn Trp Arg Glu Thr Asp Leu Leu Arg Arg Gln Arg His Asp Asn Ala Gln Leu Leu Thr Ala Ile Asp
GAT ACT TTG GCC TCA TTT GGA AAC TGG AGA GAG ACA GAC TTG CTA AGG CGC CAA AGA CAT GAT AAT GCC CAG TTA CTC ACG GCC ATT GAC

                                    370                                     Ser                                     390
Phe Asp Gly Asp Thr Val Gly Leu Ala Tyr Val Gly Gly Met Cys Gln Leu Lys His Pro Thr Gly Val Ile Gln Asp His Ser Ala Ile
TTT GAT GGA GAC ACT GTA GGA TTG GCT TAT GTG GGC GGT ATG TGC CAA CTG AAG CAT CCT ACA GGA GTT ATC CAG GAT CAT AGT GCA ATA

                                    400                                     410                                     420
Asn Leu Leu Val Ala Leu Thr Met Ala His Glu Leu Gly His Asn Leu Gly Met Asn His Asp Gly Asn Gln Cys His Cys Gly Ala Asn
AAT CTT TTG GTT GCA CTT ACA ATG GCC CAT GAG CTG GGT CAT AAT CTG GGC ATG AAT CAT GAT GGA AAT CAG TGT CAT TGC GGT GCT AAC

                        Met         430                                     440                                     450
Ser Cys Val Met Ala Ala Val Leu Ser Asp Gln Pro Ser Lys Leu Phe Ser Asp Cys Ser Lys Lys Asp Tyr Gln Thr Phe Leu Thr Val
TCG TGC GTC ATG GCT GCT GTG CTA AGT GAT CAA CCC TCC AAA CTA TTC AGC GAT TGT AGT AAG AAA GAC TAT CAG ACG TTT CTT ACG GTT

                                    460                                     470                                 478
Asn Asn Pro Gln Cys Ile Leu Asn Lys Pro Leu Arg Thr Asp Thr Val Ser Thr Pro Val Ser Gly Asn Glu Leu Leu Glu Ala OP
AAT AAC CCA CAA TGC ATT CTC AAT AAA CCC TTG AGA ACA GAT ACT GTT TCA ACT CCA GTT TCT GGA AAT GAA CTT TTG GAG GCG TGA
```

FIG. 8-1

pKS308

```
1   GGATCCCCAGCTTAGTTCATAGGTCCATTCTCTTAGCGCAACTACAGAGAACAGGGGCACAAACAGGCAAAAAACGGGCACAACCTCAATGGAGTGATGCAACCTGCCTGGAGTAAATGATG
    CCTAGGGGTCGAATCAAGTATCCAGGTAAGAGAATCGCGTTGATGTCTCTTGTCCCCGTGTTTGTCCGTTTTTTGCCCGTGTTGGAGTTACCTCACTACGTTGGACGGACCTCATTTACTAC
    BamHI

123 ACACAAGGGAATTGACCCACGCATGTATCTATCTCATTTTCTTACACCTTCTATTACCTTCTGCTCTCTCTGATTTGGAAAAAGCTGAAAAAAAAGGTTGAAACCAGTTCCCTGAAATTA
    TGTGTTCCGTTAACTGGGTGCGTACATAGATAGAGTAAAAGAATGTGGAAGATAATGGAAGACGAGAGAGACTAAACCTTTTTCGACTTTTTTTTCCAACTTTGGTCAAGGGACTTTAAT

243 TTCCCCTACTTGACTAATAAGTATATAAAGACGGTAGGTATTGATTGTAATTCTGTAAATCTATTTCTTAAACTTCTTAAATTCTACTTTTATAGTTAGTCTTTTTTTTAGTTTTAAAAC
    AAGGGGATGAACTGATTATTCATATATTTCTGCCATCCATAACTAACATTAAGACATTTAGATAAAGAATTTGAAGAATTTAAGATGAAAATATCAATCAGAAAAAAAATCAAAATTTTG

                                                MetArgPheProSerIlePheThrAlaValLeuPheAlaAlaSerSerAlaLeuAlaAlaProValAsnThrThrThr
363 ACCAAGAACTTAGTTTCGAATAAACACACATAAACAAACACCATGAGATTTCCTTCAATTTTTACTGCAGTTTTATTCGCAGCATCCTCCGCATTAGCTGCTCCAGTCAACACTACAACA
    TGGTTCTTGAATCAAAGCTTATTTGTGTGTATTTGTTTGTGGTACTCTAAAGGAAGTTAAAAATGACGTCAAAATAAGCGTCGTAGGAGGCGTAATCGACGAGGTCAGTTGTGATGTTGT
                                                                  PstI

    GluAspGluThrAlaGlnIleProAlaGluAlaValIleGlyTyrLeuAspLeuGluGlyAspPheAspValAlaValLeuProPheSerAsnSerThrAsnAsnGlyLeuLeuPheIle
483 GAAGATGAAACGGCACAAATTCCGGCTGAAGCTGTCATCGGTTACTTAGATTTAGAAGGGGATTTCGATGTTGCTGTTTTGCCATTTTCCAACAGCACAAATAACGGGTTATTGTTTATA
    CTTCTACTTTGCCGTGTTTAAGGCCGACTTCGACAGTAGCCAATGAATCTAAATCTTCCCCTAAAGCTACAACGACAAAACGGTAAAAGGTTGTCGTGTTTATTGCCCAATAACAAATAT

    AsnThrThrIleAlaSerIleAlaAlaLysGluGluGlyValSerLeuAspLysArgGlnGlnArgPheProGlnArgTyrValGlnLeuValIleValAlaAspHisArgMetAsnThr
603 AATACTACTATTGCCAGCATTGCTGCTAAAGAAGAAGGGGTATCTCTAGATAAAAGACAACAAAGATTCCCCCAAAGATACGTTCAGCTGGTCATAGTTGCGGATCACAGAATGAATACG
    TTATGATGATAACGGTCGTAACGACGATTTCTTCTTCCCCATAGAGATCTATTTTCTGTTGTTTCTAAGGGGGTTTCTATGCAAGTCGACCAGTATCAACGCCTAGTGTCTTACTTATGC
                                               XbaI                                  PvuII
```

FIG. 8-2

```
     LysTyrAsnGlyAspSerAspLysIleArgGlnTrpValHisGlnIleValAsnThrIleAsnGluIleTyrArgProLeuAsnIleGlnPheThrLeuValGlyLeuGluIleTrpSer
723  AAATACAATGGTGATTCAGATAAGATAAGACAATGGGTTCACCAGATTGTCAACACTATAAATGAGATTTACAGACCTTTGAATATTCAATTCACACTGGTTGGCCTAGAAATTTGGTCC
     TTTATGTTACCACTAAGTCTATTCTATTCTGTTACCCAAGTGGTCTAACAGTTGTGATATTTACTCTAAATGTCTGGAAACTTATAAGTTAAGTGTGACCAACCGGATCTTTAAACCAGG

     AsnGlnAspLeuIleThrValThrSerValSerHisAspThrLeuAlaSerPheGlyAsnTrpArgGluThrAspLeuLeuArgArgGlnArgHisAspAsnAlaGlnLeuLeuThrAla
843  AACCAAGATTTGATTACCGTGACATCAGTATCACATGATACTTTGGCCTCATTTGGAAACTGGAGAGAGACAGACTTGCTAAGGCGCCAAAGACATGATAATGCCCAGTTACTCACGGCC
     TTGGTTCTAAACTAATGGCACTGTAGTCATAGTGTACTATGAAACCGGAGTAAACCTTTGACCTCTCTCTGTCTGAACGATTCCGCGGTTTCTGTACTATTACGGGTCAATGAGTGCCGG

     IleAspPheAspGlyAspThrValGlyLeuAlaTyrValGlyGlyMetCysGlnLeuLysHisSerThrGlyValIleGlnAspHisSerAlaIleAsnLeuLeuValAlaLeuThrMet
963  ATTGACTTTGATGGAGACACTGTAGGATTGGCTTATGTGGGCGGTATGTGCCAACTGAAGCACTCCACTGGAGTTATCCAGGATCATAGTGCAATAAATCTTTTGGTTGCACTTACAATG
     TAACTGAAACTACCTCTGTGACATCCTAACCGAATACACCCGCCATACACGGTTGACTTCGTGAGGTGACCTCAATAGGTCCTAGTATCACGTTATTTAGAAAACCAACGTGAATGTTAC

     AlaHisGluLeuGlyHisAsnLeuGlyMetAsnHisAspGlyAsnGlnCysHisCysGlyAlaAsnSerCysValMetAlaAlaMetLeuSerAspGlnProSerLysLeuPheSerAsp
1083 GCCCATGAGCTGGGTCATAATCTGGGCATGAATCATGATGGAAATCAGTGTCATTGCGGTGCTAACTCGTGCGTCATGGCTGCCATGTTAAGTGATCAACCCTCCAAACTATTCAGCGAT
     CGGGTACTCGACCCAGTATTAGACCCGTACTTAGTACTACCTTTAGTCACAGTAACGCCACGATTGAGCACGCAGTACCGACGGTACAATTCACTAGTTGGGAGGTTTGATAAGTCGCTA

     CysSerLysLysAspTyrGlnThrPheLeuThrValAsnAsnProGlnCysIleLeuAsnLysProAM OC
1203 TGTAGTAAGAAAGACTATCAGACGTTTCTTACGGTTAATAACCCACAATGCATTCTCAATAAACCCTAGTAAGTCGACTTTGTTCCCACTGTACTTTTAGCTCGTACAAAATACAATATA
     ACATCATTCTTTCTGATAGTCTGCAAAGAATGCCAATTATTGGGTGTTACGTAAGAGTTATTTGGGATCATTCAGCTGAAACAAGGGTGACATGAAAATCGAGCATGTTTTATGTTATAT
                                                    NsiI                 SalI

1323 CTTTTCATTTCTCCGTAAACAACATGTTTTCCCATGTAATATCCTTTTCTATTTTTCGTTCCGTTACCAACTTTACACATACTTTATATAGCTATTCACTTCTATACACTAAAAAACTAA
     GAAAAGTAAAGAGGCATTTGTTGTACAAAAGGGTACATTATAGGAAAAGATAAAAAGCAAGGCAATGGTTGAAATGTGTATGAAATATATCGATAAGTGAAGATATGTGATTTTTTGATT

1443 GACAATTTTAATTTTGCTGCCTGCCATATTTCAATTTGTTATAAATTCCTATAATTTATCCTATTAGTAGCTAAAAAAAGATGAATGTGAATCGAATCCTAAGAGAATTCGGATCC
     CTGTTAAAATTAAAACGACGGACGGTATAAAGTTAAACAATATTTAAGGATATTAAATAGGATAATCATCGATTTTTTTCTACTTACACTTAGCTTAGGATTCTCTTAAGCCTAGG
                                                                                                                EcoRI  BamHI
```

FIG. 9-1

```
1   GGATCCCCAGCTTAGTTCATAGGTCCATTCTCTTAGCGCAACTACAGAGAACAGGGGCACAAACAGGCAAAAAACGGGCACAACCTCAATGGAGTGATGCAACCTGCCTGGAGTAAATGATG
    CCTAGGGGTCGAATCAAGTATCCAGGTAAGAGAATCGCGTTGATGTCTCTTGTCCCCGTGTTTGTCCGTTTTTTGCCCGTGTTGGAGTTACCTCACTACGTTGGACGGACCTCATTTACTAC
    BamHI

123 ACACAAGGCAATTGACCCACGCATGTATCTATCTCATTTTCTTACACCTTCTATTACCTTCTGCTCTCTCTGATTTGGAAAAAGCTGAAAAAAAAGGTTGAAACCAGTTCCCTGAAATTA
    TGTGTTCCGTTAACTGGGTGCGTACATAGATAGAGTAAAAGAATGTGGAAGATAATGGAAGACGAGAGAGACTAAACCTTTTTCGACTTTTTTTTCCAACTTTGGTCAAGGGACTTTAAT

243 TTCCCCTACTTGACTAATAAGTATATAAAGACGGTAGGTATTGATTGTAATTCTGTAAATCTATTTCTTAAACTTCTTAAATTCTACTTTTATAGTTAGTCTTTTTTTTAGTTTTAAAAC
    AAGGGGATGAACTGATTATTCATATATTTCTGCCATCCATAACTAACATTAAGACATTTAGATAAAGAATTTGAAGAATTTAAGATGAAAATATCAATCAGAAAAAAAATCAAAATTTTG

                                                        MetArgPheProSerIlePheThrAlaValLeuPheAlaAlaSerSerAlaLeuAlaAlaProValAsnThrThrThr
363 ACCAAGAACTTAGTTTCGAATAAACACACATAAACAAACACCATGAGATTTCCTTCAATTTTTACTGCAGTTTTATTCGCAGCATCCTCCGCATTAGCTGCTCCAGTCAACACTACAACA
    TGGTTCTTGAATCAAAGCTTATTTGTGTGTATTTGTTTGTGGTACTCTAAAGGAAGTTAAAAATGACGTCAAAATAAGCGTCGTAGGAGGCGTAATCGACGAGGTCAGTTGTGATGTTGT
                                                                        PstI

    GluAspGluThrAlaGlnIleProAlaGluAlaValIleGlyTyrLeuAspLeuGluGlyAspPheAspValAlaValLeuProPheSerAsnSerThrAsnAsnGlyLeuLeuPheIle
483 GAAGATGAAACGGCACAAATTCCGGCTGAAGCTGTCATCGGTTACTTAGATTTAGAAGGGGATTTCGATGTTGCTGTTTTGCCATTTTCCAACAGCACAAATAACGGGTTATTGTTTATA
    CTTCTACTTTGCCGTGTTTAAGGCCGACTTCGACAGTAGCCAATGAATCTAAATCTTCCCCTAAAGCTACAACGACAAAACGGTAAAAGGTTGTCGTGTTTATTGCCCAATAACAAATAT

    AsnThrThrIleAlaSerIleAlaAlaLysGluGluGlyValSerLeuAspLysArgSerSerIleIleLeuGluSerGlyAsnValAsnAspTyrGluValValTyrProArgLysVal
603 AATACTACTATTGCCAGCATTGCTGCTAAAGAAGAAGGGGTATCTCTAGATAAAAGGAGCTCTATAATCCTGGAATCTGGGAACGTTAATGATTATGAAGTAGTGTATCCACGAAAAGTC
    TTATGATGATAACGGTCGTAACGACGATTTCTTCTTCCCCATAGAGATCTATTTTCCTCGAGATATTAGGACCTTAGACCCTTGCAATTACTAATACTTCATCACATAGGTGCTTTTCAG
                                                XbaI      SacI

    ThrProValProArgGlyAlaValGlnProLysTyrGluAspAlaMetGlnTyrGluPheLysValAsnGlyGluProValValLeuHisLeuGluLysAsnLysGlyLeuPheSerGlu
723 ACTCCAGTGCCCAGAGGAGCAGTTCAGCCAAAGTATGAAGATGCCATGCAATATGAATTTAAAGTGAATGGAGAGCCAGTGGTCCTTCACCTGGAAAAAAATAAAGGACTTTTTTCAGAA
    TGAGGTCACGGGTCTCCTCGTCAAGTCGGTTTCATACTTCTACGGTACGTTATACTTAAATTTCACTTACCTCTCGGTCACCAGGAAGTGGACCTTTTTTTATTTCCTGAAAAAAGTCTT
```

FIG. 9-2

```
     AspTyrSerGluThrHisTyrSerProAspGlyArgGluIleThrThrTyrProLeuValGluAspHisCysTyrTyrHisGlyArgIleGluAsnAspAlaAspSerThrAlaSerIle
843  GATTACAGCGAGACTCATTATTCCCCTGATGGCAGAGAAATTACAACATACCCCCTGGTTGAGGATCACTGCTATTATCATGGACGCATCGAGAATGATGCTGACTCAACTGCAAGCATC
     CTAATGTCGCTCTGAGTAATAAGGGGACTACCGTCTCTTTAATGTTGTATGGGGGACCAACTCCTAGTGACGATAATAGTACCTGCGTAGCTCTTACTACGACTGAGTTGACGTTCGTAG

     SerAlaCysAsnGlyLeuLysGlyHisPheLysLeuGlnGlyGluMetTyrLeuIleGluProLeuGluLeuSerAspSerGluAlaHisAlaValTyrLysTyrGluAsnValGluLys
963  AGTGCATGCAACGGTTTGAAAGGACATTTCAAGCTTCAAGGGGAGATGTACCTTATTGAACCGTTGGAGCTTTCCGACAGTGAAGCCCATGCAGTCTACAAATATGAAAATGTAGAAAAA
     TCACGTACGTTGCCAAACTTTCCTGTAAAGTTCGAAGTTCCCCTCTACATGGAATAACTTGGCAACCTCGAAAGGCTGTCACTTCGGGTACGTCAGATGTTTATACTTTTACATCTTTTT
                                   HinDIII

     GluAspGluAlaProLysMetCysGlyValThrGlnAsnTrpGluSerTyrGluProIleLysLysAlaPheGlnLeuAsnLeuThrProGluGlnGlnArgPheProGlnArgTyrVal
1083 GAGGATGAGGCCCCCAAAATGTGTGGGGTAACCCAGAATTGGGAATCATATGAGCCCATCAAAAAGGCCTTTCAGTTAAATCTTACTCCTGAACAACAAAGGTTCCCCCAAAGATATGTT
     CTCCTACTCCGGGGGTTTTACACACCCCATTGGGTCTTAACCCTTAGTATACTCGGGTAGTTTTTCCGGAAAGTCAATTTAGAATGAGGACTTGTTGTTTCCAAGGGGGTTTCTATACAA
                               BstEII

     GlnLeuValIleValAlaAspHisArgMetTyrMetLysTyrAsnAsnAspSerAsnLeuIleArgGlnTrpValHisGlnIleValAsnThrIleAsnGluIleTyrArgProLeuAsn
1203 CAGCTTGTCATAGTTGCAGATCACAGAATGTACATGAAATACAATAATGATTCAAATTTGATAAGACAATGGGTACATCAAATTGTCAACACTATAAATGAGATTTACAGACCTTTGAAT
     GTCGAACAGTATCAACGTCTAGTGTCTTACATGTACTTTATGTTATTACTAAGTTTAAACTATTCTGTTACCCATGTAGTTTAACAGTTGTGATATTTACTCTAAATGTCTGGAAACTTA

     IleGlnPheThrLeuValGlyLeuGluIleTrpSerAsnGlnAspLeuIleThrValThrSerValSerHisAspThrLeuAlaSerPheGlyAsnTrpArgGluThrAspLeuLeuArg
1323 ATTCAATTCACACTGGTTGGCCTAGAAATTTGGTCCAACCAAGATTTGATTACCGTGACATCAGTATCACATGATACTTTGGCCTCATTTGGAAACTGGAGAGAGACAGACTTGCTAAGG
     TAAGTTAAGTGTGACCAACCGGATCTTTAAACCAGGTTGGTTCTAAACTAATGGCACTGTAGTCATAGTGTACTATGAAACCGGAGTAAACCTTTGACCTCTCTCTGTCTGAACGATTCC

     ArgGlnArgHisAspAsnAlaGlnLeuLeuThrAlaIleAspPheAspGlyAspThrValGlyLeuAlaTyrValGlyGlyMetCysGlnLeuLysHisProThrGlyValIleGlnAsp
1443 CGCCAAAGACATGATAATGCCCAGTTACTCACGGCCATTGACTTTGATGGAGACACTGTAGGATTGGCTTATGTGGGCGGTATGTGCCAACTGAAGCATCCTACAGGAGTTATCCAGGAT
     GCGGTTTCTGTACTATTACGGGTCAATGAGTGCCGGTAACTGAAACTACCTCTGTGACATCCTAACCGAATACACCCGCCATACACGGTTGACTTCGTAGGATGTCCTCAATAGGTCCTA
```

FIG. 9-3

HisSerAlaIleAsnLeuLeuValAlaLeuThrMetAlaHisGluLeuGlyHisAsnLeuGlyMetAsnHisAspGlyAsnGlnCysHisCysGlyAlaAsnSerCysValMetAlaAla
1563 CATAGTGCAATAAATCTTTTGGTTGCACTTACAATGGCCCATGAGCTGGGTCATAATCTGGGCATGAATCATGATGGAAATCAGTGTCATTGCGGTGCTAACTCGTGCGTCATGGCTGCT
GTATCACGTTATTTAGAAAACCAACGTGAATGTTACCGGGTACTCGACCCAGTATTAGACCCGTACTTAGTACTACCTTTAGTCACAGTAACGCCACGATTGAGCACGCAGTACCGACGA

ValLeuSerAspGlnProSerLysLeuPheSerAspCysSerLysLysAspTyrGlnThrPheLeuThrValAsnAsnProGlnCysIleLeuAsnLysProLeuArgThrAspThrVal
1683 GTGCTAAGTGATCAACCCTCCAAACTATTCAGCGATTGTAGTAAGAAAGACTATCAGACGTTTCTTACGGTTAATAACCCACAATGCATTCTCAATAAACCCTTGAGAACAGATACTGTT
CACGATTCACTAGTTGGGAGGTTTGATAAGTCGCTAACATCATTCTTTCTGATAGTCTGCAAAGAATGCCAATTATTGGGTGTTACGTAAGAGTTATTTGGGAACTCTTGTCTATGACAA
NsiI

SerThrProValSerGlyAsnGluLeuLeuGluAlaOP
1803 TCAACTCCAGTTTCTGGAAATGAACTTTTGGAGGCGTGAGAAGAATGTGACTGTGGCTCTCCTGCAGGTCGACTTGGTTGAACACGTTGCCAAGGCTTAAGTGAATTTACTTTAAAGTCT
AGTTGAGGTCAAAGACCTTTACTTGAAAACCTCCGCACTCTTCTTACACTGACACCGAGAGGACGTCCAGCTGAACCAACTTGTGCAACGGTTCCGAATTCACTTAAATGAAATTTCAGA
PstI SalI

1923 TGCATTTAAATAAATTTTCTTTTTATAGCTTTATGACTTAGTTTCAATTTATATACTATTTTAATGACATTTTCGATTCATTGATTGAAAGCTTTGTGTTTTTTCTTGATGCGCTATTGC
ACGTAAATTTATTTAAAAGAAAAATATCGAAATACTGAATCAAAGTTAAATATATGATAAAATTACTGTAAAAGCTAAGTAACTAACTTTCGAAACACAAAAAAGAACTACGCGATAACG
HinDIII

2043 ATTGTTCTTGTCTTTTTCGCCACATGTAATATCTGTAGTAGATACCTGATACATTGTGGATGCTGAGTGAAATTTTAGTTAATAATGGAGGCGCTCTTAATAATTTTGGGGATATTGGCT
TAACAAGAACAGAAAAAGCGGTGTACATTATAGACATCATCTATGGACTATGTAACACCTACGACTCACTTTAAAATCAATTATTACCTCCGCGAGAATTATTAAAACCCCTATAACCGA

2163 TTTTTTTTTAAAGTTTACAAATGAATTTTTTCCGCCAGGATAACGATTCTGAAGTTACTCTTAGCGTTCCTATCGGTACAGCCATCAAATCATGCCTATAAATCATGCCTATATTTGCGT
AAAAAAAAATTTCAAATGTTTACTTAAAAAAGGCGGTCCTATTGCTAAGACTTCAATGAGAATCGCAAGGATAGCCATGTCGGTAGTTTAGTACGGATATTTAGTACGGATATAAACGCA

```
2283 GCAGTCAGTATCATCTACATGAAAAAAACTCCCGCAATTTCTTATAGAATACGTTGAAAATTAAATGTACGCGCCAAGATAAGATAACATATATCTAGCTAGATGCAGTAATATACACAG
     CGTCAGTCATAGTAGATGTACTTTTTTTGAGGGCGTTAAAGAATATCTTATGCAACTTTTAATTTACATGCGCGGTTCTATTCTATTGTATATAGATCGATCTACGTCATTATATGTGTC

2403 ATTCCCGCGGACGTGGGAAGGAAAAAATTAGATAACAAAATCTGAGTGATATGGAAATTCCGCTGTATAGCTCATATCTTTCCCTTCAACACCAGAAATGTAAAAATCTTGTTACGAAGG
     TAAGGGCGCCTGCACCCTTCCTTTTTTAATCTATTGTTTTAGACTCACTATACCTTTAAGGCGACATATCGAGTATAGAAAGGGAAGTTGTGGTCTTTACATTTTTAGAACAATGCTTCC
         SacII

2523 ATCTTTTTGCTAATGTTTCTCGCTCAATCCTCATTTCTTCCCTACGAAGAGTCAAATCTACTTGTTTTCTGCCGGTATCAAGATCCATATCTTCTAGTTTCACCATCAAAGTCCAATTTC
     TAGAAAAACGATTACAAAGAGCGAGTTAGGAGTAAAGAAGGGATGCTTCTCAGTTTAGATGAACAAAAGACGGCCATAGTTCTAGGTATAGAAGATCAAAGTGGTAGTTTCAGGTTAAAG

2643 TAGTATACAGTTTATGTCCCAACGTAACAGACAATCAAAATTGGAAAGGATAAGTATCCTTCAAAGAATGATTCTGCGCTGGCTCCTGAACCGCCTAATGGGAACAGAGAAGTCCAAAAC
     ATCATATGTCAAATACAGGGTTGCATTGTCTGTTAGTTTTAACCTTTCCTATTCATAGGAAGTTTCTTACTAAGACGCGACCGAGGACTTGGCGGATTACCCTTGTCTCTTCAGGTTTTG

2763 GATGCTATAAGAACCAGAAATAAAACGATAAAACCATACCAGGATCC
     CTACGATATTCTTGGTCTTTATTTTGCTATTTTGGTATGGTCCTAGG
                                                BamHI
```

FIG. 9-4
pKS 311

FIG. 10-1

```
1    GGATCCCCAGCTTAGTTCATAGGTCCATTCTCTTAGCGCAACTACAGAGAACAGGGGCACAAACAGGCAAAAAACGGGCACAACCTCAATGGAGTGATGCAACCTGCCTGGAGTAAATGATG
     CCTAGGGGTCGAATCAAGTATCCAGGTAAGAGAATCGCGTTGATGTCTCTTGTCCCCGTGTTTGTCCGTTTTTTGCCCGTGTTGGAGTTACCTCACTACGTTGGACGGACCTCATTTACTAC
     BamHI

123  ACACAAGGCAATTGACCCACGCATGTATCTATCTCATTTTCTTACACCTTCTATTACCTTCTGCTCTCTCTGATTTGGAAAAAGCTGAAAAAAAAGGTTGAAACCAGTTCCCTGAAATTA
     TGTGTTCCGTTAACTGGGTGCGTACATAGATAGAGTAAAAGAATGTGGAAGATAATGGAAGACGAGAGAGACTAAACCTTTTTCGACTTTTTTTTCCAACTTTGGTCAAGGGACTTTAAT

243  TTCCCCTACTTGACTAATAAGTATATAAAGACGGTAGGTATTGATTGTAATTCTGTAAATCTATTTCTTAAACTTCTTAAATTCTACTTTTATAGTTAGTCTTTTTTTTAGTTTTAAAAC
     AAGGGGATGAACTGATTATTCATATATTTCTGCCATCCATAACTAACATTAAGACATTTAGATAAAGAATTTGAAGAATTTAAGATGAAAATATCAATCAGAAAAAAAATCAAAATTTTG

                                                                 MetArgPheProSerIlePheThrAlaValLeuPheAlaAlaSerSerAlaLeuAlaAlaProValAsnThrThrThr
363  ACCAAGAACTTAGTTTCGAATAAACACACATAAACAAACACCATGAGATTTCCTTCAATTTTTACTGCAGTTTTATTCGCAGCATCCTCCGCATTAGCTGCTCCAGTCAACACTACAACA
     TGGTTCTTGAATCAAAGCTTATTTGTGTGTATTTGTTTGTGGTACTCTAAAGGAAGTTAAAAATGACGTCAAAATAAGCGTCGTAGGAGGCGTAATCGACGAGGTCAGTTGTGATGTTGT
                                                                      PstI

     GluAspGluThrAlaGlnIleProAlaGluAlaValIleGlyTyrLeuAspLeuGluGlyAspPheAspValAlaValLeuProPheSerAsnSerThrAsnAsnGlyLeuLeuPheIle
483  GAAGATGAAACGGCACAAATTCCGGCTGAAGCTGTCATCGGTTACTTAGATTTAGAAGGGGATTTCGATGTTGCTGTTTTGCCATTTTCCAACAGCACAAATAACGGGTTATTGTTTATA
     CTTCTACTTTGCCGTGTTTAAGGCCGACTTCGACAGTAGCCAATGAATCTAAATCTTCCCCTAAAGCTACAACGACAAAACGGTAAAAGGTTGTCGTGTTTATTGCCCAATAACAAATAT

     AsnThrThrIleAlaSerIleAlaAlaLysGluGluGlyValSerLeuAspLysArgSerSerIleIleLeuGluSerGlyAsnValAsnAspTyrGluValValTyrProArgLysVal
603  AATACTACTATTGCCAGCATTGCTGCTAAAGAAGAAGGGGTATCTCTAGATAAAAGGAGCTCTATAATCCTGGAATCTGGGAACGTTAATGATTATGAAGTAGTGTATCCACGAAAAGTC
     TTATGATGATAACGGTCGTAACGACGATTTCTTCTTCCCCATAGAGATCTATTTTCCTCGAGATATTAGGACCTTAGACCCTTGCAATTACTAATACTTCATCACATAGGTGCTTTTCAG
                                                 XbaI       SacI

     ThrProValProArgGlyAlaValGlnProLysTyrGluAspAlaMetGlnTyrGluPheLysValAsnGlyGluProValValLeuHisLeuGluLysAsnLysGlyLeuPheSerGlu
723  ACTCCAGTGCCCAGAGGAGCAGTTCAGCCAAAGTATGAAGATGCCATGCAATATGAATTTAAAGTGAATGGAGAGCCAGTGGTCCTTCACCTGGAAAAAAATAAAGGACTTTTTTCAGAA
     TGAGGTCACGGGTCTCCTCGTCAAGTCGGTTTCATACTTCTACGGTACGTTATACTTAAATTTCACTTACCTCTCGGTCACCAGGAAGTGGACCTTTTTTTATTTCCTGAAAAAAGTCTT
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FIG. 10-2

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     AspTyrSerGluThrHisTyrSerProAspGlyArgGluIleThrThrTyrProLeuValGluAspHisCysTyrTyrHisGlyArgIleGluAsnAspAlaAspSerThrAlaSerIle
843  GATTACAGCGAGACTCATTATTCCCCTGATGGCAGAGAAATTACAACATACCCCCTGGTTGAGGATCACTGCTATTATCATGGACGCATCGAGAATGATGCTGACTCAACTGCAAGCATC
     CTAATGTCGCTCTGAGTAATAAGGGGACTACCGTCTCTTTAATGTTGTATGGGGGACCAACTCCTAGTGACGATAATAGTACCTGCGTAGCTCTTACTACGACTGAGTTGACGTTCGTAG

     SerAlaCysAsnGlyLeuLysGlyHisPheLysLeuGlnGlyGluMetTyrLeuIleGluProLeuGluLeuSerAspSerGluAlaHisAlaValTyrLysTyrGluAsnValGluLys
963  AGTGCATGCAACGGTTTGAAAGGACATTTCAAGCTTCAAGGGGAGATGTACCTTATTGAACCGTTGGAGCTTTCCGACAGTGAAGCCCATGCAGTCTACAAATATGAAAATGTAGAAAAA
     TCACGTACGTTGCCAAACTTTCCTGTAAAGTTCGAAGTTCCCCTCTACATGGAATAACTTGGCAACCTCGAAAGGCTGTCACTTCGGGTACGTCAGATGTTTATACTTTTACATCTTTTT
                                   HinDIII

     GluAspGluAlaProLysMetCysGlyValThrGlnAsnTrpGluSerTyrGluProIleLysLysAlaPheGlnLeuAsnLeuThrLysArgGlnGlnArgPheProGlnArgTyrVal
1083 GAGGATGAGGCCCCCAAAATGTGTGGGGTAACCCAGAATTGGGAATCATATGAGCCCATCAAAAAGGCCTTTCAGTTAAATCTTACTAAAAGACAACAAAGATTCCCCCAAAGATACGTT
     CTCCTACTCCGGGGGTTTTACACACCCCATTGGGTCTTAACCCTTAGTATACTCGGGTAGTTTTTCCGGAAAGTCAATTTAGAATGATTTTCTGTTGTTTCTAAGGGGGTTTCTATGCAA
                                BstEII

     GlnLeuValIleValAlaAspHisArgMetAsnThrLysTyrAsnGlyAspSerAspLysIleArgGlnTrpValHisGlnIleValAsnThrIleAsnGluIleTyrArgProLeuAsn
1203 CAGCTGGTCATAGTTGCGGATCACAGAATGAATACGAAATACAATGGTGATTCAGATAAGATAAGACAATGGGTTCACCAGATTGTCAACACTATAAATGAGATTTACAGACCTTTGAAT
     GTCGACCAGTATCAACGCCTAGTGTCTTACTTATGCTTTATGTTACCACTAAGTCTATTCTATTCTGTTACCCAAGTGGTCTAACAGTTGTGATATTTACTCTAAATGTCTGGAAACTTA
     PvuII

     IleGlnPheThrLeuValGlyLeuGluIleTrpSerAsnGlnAspLeuIleThrValThrSerValSerHisAspThrLeuAlaSerPheGlyAsnTrpArgGluThrAspLeuLeuArg
1323 ATTCAATTCACACTGGTTGGCCTAGAAATTTGGTCCAACCAAGATTTGATTACCGTGACATCAGTATCACATGATACTTTGGCCTCATTTGGAAACTGGAGAGAGACAGACTTGCTAAGG
     TAAGTTAAGTGTGACCAACCGGATCTTTAAACCAGGTTGGTTCTAAACTAATGGCACTGTAGTCATAGTGTACTATGAAACCGGAGTAAACCTTTGACCTCTCTCTGTCTGAACGATTCC

     ArgGlnArgHisAspAsnAlaGlnLeuLeuThrAlaIleAspPheAspGlyAspThrValGlyLeuAlaTyrValGlyGlyMetCysGlnLeuLysHisSerThrGlyValIleGlnAsp
1443 CGCCAAAGACATGATAATGCCCAGTTACTCACGGCCATTGACTTTGATGGAGACACTGTAGGATTGGCTTATGTGGGCGGTATGTGCCAACTGAAGCACTCCACTGGAGTTATCCAGGAT
     GCGGTTTCTGTACTATTACGGGTCAATGAGTGCCGGTAACTGAAACTACCTCTGTGACATCCTAACCGAATACACCCGCCATACACGGTTGACTTCGTGAGGTGACCTCAATAGGTCCTA
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     HisSerAlaIleAsnLeuLeuValAlaLeuThrMetAlaHisGluLeuGlyHisAsnLeuGlyMetAsnHisAspGlyAsnGlnCysHisCysGlyAlaAsnSerCysValMetAlaAla
1563 CATAGTGCAATAAATCTTTTGGTTGCACTTACAATGGCCCATGAGCTGGGTCATAATCTGGGCATGAATCATGATGGAAATCAGTGTCATTGCGGTGCTAACTCGTGCGTCATGGCTGCC
     GTATCACGTTATTTAGAAAACCAACGTGAATGTTACCGGGTACTCGACCCAGTATTAGACCCGTACTTAGTACTACCTTTAGTCACAGTAACGCCACGATTGAGCACGCAGTACCGACGG

     MetLeuSerAspGlnProSerLysLeuPheSerAspCysSerLysLysAspTyrGlnThrPheLeuThrValAsnAsnProGlnCysIleLeuAsnLysProAM OC
1683 ATGTTAAGTGATCAACCCTCCAAACTATTCAGCGATTGTAGTAAGAAAGACTATCAGACGTTTCTTACGGTTAATAACCCACAATGCATTCTCAATAAACCCTAGTAAGTCGACTTTGTT
     TACAATTCACTAGTTGGGAGGTTTGATAAGTCGCTAACATCATTCTTTCTGATAGTCTGCAAAGAATGCCAATTATTGGGTGTTACGTAAGAGTTATTTGGGATCATTCAGCTGAAACAA
                                                                                           NsiI                  SalI

1803 CCCACTGTACTTTTAGCTCGTACAAAATACAATATACTTTTCATTTCTCCGTAAACAACATGTTTTCCCATGTAATATCCTTTTCTATTTTTCGTTCCGTTACCAACTTTACACATACTT
     GGGTGACATGAAAATCGAGCATGTTTTATGTTATATGAAAAGTAAAGAGGCATTTGTTGTACAAAAGGGTACATTATAGGAAAAGATAAAAAGCAAGGCAATGGTTGAAATGTGTATGAA

1923 TATATAGCTATTCACTTCTATACACTAAAAAACTAAGACAATTTTAATTTTGCTGCCTGCCATATTTCAATTTGTTATAAATTCCTATAATTTATCCTATTAGTAGCTAAAAAAAGATGA
     ATATATCGATAAGTGAAGATATGTGATTTTTTGATTCTGTTAAAATTAAAACGACGGACGGTATAAAGTTAAACAATATTTAAGGATATTAAATAGGATAATCATCGATTTTTTTCTACT

2043 ATGTGAATCGAATCCTAAGAGAATTCGGATCC
     TACACTTAGCTTAGGATTCTCTTAAGCCTAGG
                         EcoRI  BamHI
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FIG. 10-3

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FIG. 11-1

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1   GGATCCCCAGCTTAGTTCATAGGTCCATTCTCTTAGCGCAACTACAGAGAACAGGGGCACAAACAGGCAAAAAACGGGCACAACCTCAATGGAGTGATGCAACCTGCCTGGAGTAAATGATG
    CCTAGGGGTCGAATCAAGTATCCAGGTAAGAGAATCGCGTTGATGTCTCTTGTCCCCGTGTTTGTCCGTTTTTTGCCCGTGTTGGAGTTACCTCACTACGTTGGACGGACCTCATTTACTAC
    BamHI

123 ACACAAGGCAATTGACCCACGCATGTATCTATCTCATTTTCTTACACCTTCTATTACCTTCTGCTCTCTCTGATTTGGAAAAAGCTGAAAAAAAAGGTTGAAACCAGTTCCCTGAAATTA
    TGTGTTCCGTTAACTGGGTGCGTACATAGATAGAGTAAAAGAATGTGGAAGATAATGGAAGACGAGAGAGACTAAACCTTTTTCGACTTTTTTTTCCAACTTTGGTCAAGGGACTTTAAT

243 TTCCCCTACTTGACTAATAAGTATATAAAGACGGTAGGTATTGATTGTAATTCTGTAAATCTATTTCTTAAACTTCTTAAATTCTACTTTTATAGTTAGTCTTTTTTTTAGTTTTAAAAC
    AAGGGGATGAACTGATTATTCATATATTTCTGCCATCCATAACTAACATTAAGACATTTAGATAAAGAATTTGAAGAATTTAAGATGAAAATATCAATCAGAAAAAAAATCAAAATTTTG

                                                  MetArgPheProSerIlePheThrAlaValLeuPheAlaAlaSerSerAlaLeuAlaAlaProValAsnThrThrThr
363 ACCAAGAACTTAGTTTCGAATAAACACACATAAACAAACACCATGAGATTTCCTTCAATTTTTACTGCAGTTTTATTCGCAGCATCCTCCGCATTAGCTGCTCCAGTCAACACTACAACA
    TGGTTCTTGAATCAAAGCTTATTTGTGTGTATTTGTTTGTGGTACTCTAAAGGAAGTTAAAAATGACGTCAAAATAAGCGTCGTAGGAGGCGTAATCGACGAGGTCAGTTGTGATGTTGT
                                                                     PstI

    GluAspGluThrAlaGlnIleProAlaGluAlaValIleGlyTyrLeuAspLeuGluGlyAspPheAspValAlaValLeuProPheSerAsnSerThrAsnAsnGlyLeuLeuPheIle
483 GAAGATGAAACGGCACAAATTCCGGCTGAAGCTGTCATCGGTTACTTAGATTTAGAAGGGGATTTCGATGTTGCTGTTTTGCCATTTTCCAACAGCACAAATAACGGGTTATTGTTTATA
    CTTCTACTTTGCCGTGTTTAAGGCCGACTTCGACAGTAGCCAATGAATCTAAATCTTCCCCTAAAGCTACAACGACAAAACGGTAAAAGGTTGTCGTGTTTATTGCCCAATAACAAATAT

    AsnThrThrIleAlaSerIleAlaAlaLysGluGluGlyValSerLeuAspSerSerIleIleLeuGluSerGlyAsnValAsnAspTyrGluValValTyrProArgLysValThrPro
603 AATACTACTATTGCCAGCATTGCTGCTAAAGAAGAAGGGGTATCTCTAGATAGCTCTATAATCCTGGAATCTGGGAACGTTAATGATTATGAAGTAGTGTATCCACGAAAAGTCACTCCA
    TTATGATGATAACGGTCGTAACGACGATTTCTTCTTCCCCATAGAGATCTATCGAGATATTAGGACCTTAGACCCTTGCAATTACTAATACTTCATCACATAGGTGCTTTTCAGTGAGGT
                                                XbaI

    ValProArgGlyAlaValGlnProLysTyrGluAspAlaMetGlnTyrGluPheLysValAsnGlyGluProValValLeuHisLeuGluLysAsnLysGlyLeuPheSerGluAspTyr
723 GTGCCCAGAGGAGCAGTTCAGCCAAAGTATGAAGATGCCATGCAATATGAATTTAAAGTGAATGGAGAGCCAGTGGTCCTTCACCTGGAAAAAAATAAAGGACTTTTTTCAGAAGATTAC
    CACGGGTCTCCTCGTCAAGTCGGTTTCATACTTCTACGGTACGTTATACTTAAATTTCACTTACCTCTCGGTCACCAGGAAGTGGACCTTTTTTTATTTCCTGAAAAAAGTCTTCTAATG
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FIG. 11-2

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     SerGluThrHisTyrSerProAspGlyArgGluIleThrThrTyrProLeuValGluAspHisCysTyrTyrHisGlyArgIleGluAsnAspAlaAspSerThrAlaSerIleSerAla
843  AGCGAGACTCATTATTCCCCTGATGGCAGAGAAATTACAACATACCCCCTGGTTGAGGATCACTGCTATTATCATGGACGCATCGAGAATGATGCTGACTCAACTGCAAGCATCAGTGCA
     TCGCTCTGAGTAATAAGGGGACTACCGTCTCTTTAATGTTGTATGGGGGACCAACTCCTAGTGACGATAATAGTACCTGCGTAGCTCTTACTACGACTGAGTTGACGTTCGTAGTCACGT

     CysAsnGlyLeuLysGlyHisPheLysLeuGlnGlyGluMetTyrLeuIleGluProLeuGluLeuSerAspSerGluAlaHisAlaValTyrLysTyrGluAsnValGluLysGluAsp
963  TGCAACGGTTTGAAAGGACATTTCAAGCTTCAAGGGGAGATGTACCTTATTGAACCGTTGGAGCTTTCCGACAGTGAAGCCCATGCAGTCTACAAATATGAAAATGTAGAAAAAGAGGAT
     ACGTTGCCAAACTTTCCTGTAAAGTTCGAAGTTCCCCTCTACATGGAATAACTTGGCAACCTCGAAAGGCTGTCACTTCGGGTACGTCAGATGTTTATACTTTTACATCTTTTTCTCCTA
                             HinDIII

     GluAlaProLysMetCysGlyValThrGlnAsnTrpGluSerTyrGluProIleLysLysAlaPheGlnLeuAsnLeuThrLysArgGlnGlnArgPheProGlnArgTyrValGlnLeu
1083 GAGGCCCCCAAAATGTGTGGGGTAACCCAGAATTGGGAATCATATGAGCCCATCAAAAAGGCCTTTCAGTTAAATCTTACTAAAAGACAACAAAGATTCCCCCAAAGATACGTTCAGCTG
     CTCCGGGGGTTTTACACACCCCATTGGGTCTTAACCCTTAGTATACTCGGGTAGTTTTTCCGGAAAGTCAATTTAGAATGATTTTCTGTTGTTTCTAAGGGGGTTTCTATGCAAGTCGAC
                         BstEII                                                                                       PvuII

     ValIleValAlaAspHisArgMetAsnThrLysTyrAsnGlyAspSerAspLysIleArgGlnTrpValHisGlnIleValAsnThrIleAsnGluIleTyrArgProLeuAsnIleGln
1203 GTCATAGTTGCGGATCACAGAATGAATACGAAATACAATGGTGATTCAGATAAGATAAGACAATGGGTTCACCAGATTGTCAACACTATAAATGAGATTTACAGACCTTTGAATATTCAA
     CAGTATCAACGCCTAGTGTCTTACTTATGCTTTATGTTACCACTAAGTCTATTCTATTCTGTTACCCAAGTGGTCTAACAGTTGTGATATTTACTCTAAATGTCTGGAAACTTATAAGTT

     PheThrLeuValGlyLeuGluIleTrpSerAsnGlnAspLeuIleThrValThrSerValSerHisAspThrLeuAlaSerPheGlyAsnTrpArgGluThrAspLeuLeuArgArgGln
1323 TTCACACTGGTTGGCCTAGAAATTTGGTCCAACCAAGATTTGATTACCGTGACATCAGTATCACATGATACTTTGGCCTCATTTGGAAACTGGAGAGAGACAGACTTGCTAAGGCGCCAA
     AAGTGTGACCAACCGGATCTTTAAACCAGGTTGGTTCTAAACTAATGGCACTGTAGTCATAGTGTACTATGAAACCGGAGTAAACCTTTGACCTCTCTCTGTCTGAACGATTCCGCGGTT

     ArgHisAspAsnAlaGlnLeuLeuThrAlaIleAspPheAspGlyAspThrValGlyLeuAlaTyrValGlyGlyMetCysGlnLeuLysHisSerThrGlyValIleGlnAspHisSer
1443 AGACATGATAATGCCCAGTTACTCACGGCCATTGACTTTGATGGAGACACTGTAGGATTGGCTTATGTGGGCGGTATGTGCCAACTGAAGCACTCCACTGGAGTTATCCAGGATCATAGT
     TCTGTACTATTACGGGTCAATGAGTGCCGGTAACTGAAACTACCTCTGTGACATCCTAACCGAATACACCCGCCATACACGGTTGACTTCGTGAGGTGACCTCAATAGGTCCTAGTATCA
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     AlaIleAsnLeuLeuValAlaLeuThrMetAlaHisGluLeuGlyHisAsnLeuGlyMetAsnHisAspGlyAsnGlnCysHisCysGlyAlaAsnSerCysValMetAlaAlaMetLeu
1563 GCAATAAATCTTTTGGTTGCACTTACAATGGCCCATGAGCTGGGTCATAATCTGGGCATGAATCATGATGGAAATCAGTGTCATTGCGGTGCTAACTCGTGCGTCATGGCTGCCATGTTA
     CGTTATTTAGAAAACCAACGTGAATGTTACCGGGTACTCGACCCAGTATTAGACCCGTACTTAGTACTACCTTTAGTCACAGTAACGCCACGATTGAGCACGCAGTACCGACGGTACAAT

     SerAspGlnProSerLysLeuPheSerAspCysSerLysLysAspTyrGlnThrPheLeuThrValAsnAsnProGlnCysIleLeuAsnLysProAM OC
1683 AGTGATCAACCCTCCAAACTATTCAGCGATTGTAGTAAGAAAGACTATCAGACGTTTCTTACGGTTAATAACCCACAATGCATTCTCAATAAACCCTAGTAAGTCGACTTTGTTCCCACT
     TCACTAGTTGGGAGGTTTGATAAGTCGCTAACATCATTCTTTCTGATAGTCTGCAAAGAATGCCAATTATTGGGTGTTACGTAAGAGTTATTTGGGATCATTCAGCTGAAACAAGGGTGA
                                                                                     NsiI                 SalI

1803 GTACTTTTAGCTCGTACAAAATACAATATACTTTTCATTTCTCCGTAAACAACATGTTTTCCCATGTAATATCCTTTTCTATTTTTCGTTCCGTTACCAACTTTACACATACTTTATATA
     CATGAAAATCGAGCATGTTTTATGTTATATGAAAAGTAAAGAGGCATTTGTTGTACAAAAGGGTACATTATAGGAAAAGATAAAAAGCAAGGCAATGGTTGAAATGTGTATGAAATATAT

1923 GCTATTCACTTCTATACACTAAAAAACTAAGACAATTTTAATTTTGCTGCCTGCCATATTTCAATTTGTTATAAATTCCTATAATTTATCCTATTAGTAGCTAAAAAAAGATGAATGTGA
     CGATAAGTGAAGATATGTGATTTTTTGATTCTGTTAAAATTAAAACGACGGACGGTATAAAGTTAAACAATATTTAAGGATATTAAATAGGATAATCATCGATTTTTTTCTACTTACACT

2043 ATCGAATCCTAAGAGAATTCGGATCC
     TAGCTTAGGATTCTCTTAAGCCTAGG
                   EcoRI  BamHI
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FIG. 11-3

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PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application number
EP 88311924.0

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
P,A	CHEMICAL ABSTRACTS vol. 108, no. 13, 28th March 1988; abstract no. 108585g; NED. B. EGEN et al.: "Isolation by preparative isoelectric focusing of a direct acting fibrinolytic enzyme from the venom of Agkistrodon contortrix contortrix (southern copperhead)"; & TOXICON 1987, vol. 25, no. 11, pages 1189-1198	1-17	C12N15/00 C12N9/64 A61K37/00
A	CHEMICAL ABSTRACTS vol. 94, no. 19, 11th May 1981; abstract no. 152588b; HUBERT PIRKLE et al.: "The primary structure of crotalase, a thrombin-like venom enzyme, exhibits closer	1-17	

TECHNICAL FIELDS SEARCHED (Int. Cl.4)

C12N15/00
C12N9/64
A61K37/00

INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims.

Claims searched completely: 1-18
Claims searched incompletely:
Claims not searched: 19
Reason for the limitation of the search: Article 52(4) EPC
Method for treatment of the human or animal body by surgery or therapy

Place of search	Date of completion of the search	Examiner
BERLIN	20.03.1989	P. JULIA Y BALLBE

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document
T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons
& : member of the same patent family, corresponding document

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application number EP88311924.0

- page 2 -

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
	homology to kallikrein than to other serine proteases"; & BIOCHEM. BIOPHYS. RES. COMMUN., 1981, vol. 99, no. 2, pages 715-721		
D,A	US - A - 4610879 (TRANCIS S. MARKLAND et al.) * whole document *	1-18	
A	EP - A - 0123544 (GENENTECH., INC.) * whole document *	5-14	TECHNICAL FIELDS SEARCHED (Int. Cl.4)